Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

SHOUT
SHARE
IT

84B
27

UNITED STATES DEPARTMENT OF AGRICULTURE
BULLETIN No. 227
Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER August 23, 1915.

THE TOXICITY TO FUNGI OF VARIOUS OILS AND SALTS, PARTICULARLY THOSE USED IN WOOD PRESERVATION

By

C. J. HUMPHREY, Assistant Pathologist, and RUTH M. FLEMING, Scientific Assistant, Office of Investigations in Forest Pathology

CONTENTS

	Page
Introduction	1
Historical	2
Tests Conducted at the Forest-Products Laboratory	14
Toxicity to Fungi of the More Important Preservatives	31
Summary	35
Bibliography	37

WASHINGTON
GOVERNMENT PRINTING OFFICE
1915

UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 227

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER August 23, 1915.

THE TOXICITY TO FUNGI OF VARIOUS OILS AND SALTS, PARTICULARLY THOSE USED IN WOOD PRESERVATION.[1]

By C. J. Humphrey, *Assistant Pathologist*, and Ruth M. Fleming, *Scientific Assistant, Office of Investigations in Forest Pathology.*

CONTENTS.

	Page.		Page.
Introduction	1	Toxicity to fungi of the more important preservatives	31
Historical	2	Summary	35
Tests conducted at the Forest-Products Laboratory	14	Bibliography	37

INTRODUCTION.

Within comparatively recent years the subject of wood preservation has become of paramount importance, largely resulting from the economic conditions which necessitate the utilization of timber inferior in its resistance to decay to species formerly readily obtained. The rise of wood preservation in the United States within the last two decades has been very rapid. The principal preservatives used have consisted of coal-tar creosote and zinc chlorid, either alone or in combination. Such experimental work as has been done prior to the last two or three years has been directed in large part toward perfecting the mechanical processes of injecting the preservatives into wood, with an idea of securing the greatest relative efficiency as compared with the cost involved—purely an engineering proposition based on

[1] The writers wish to thank Mr. Howard F. Weiss, Director of the Forest-Products Laboratory, Madison, Wis., for the interest displayed and suggestions offered during the progress of this work, as well as for the laboratory facilities placed at their disposal. Thanks are also due to Mr. Ernest Bateman, Chemist in Forest Products at the Forest-Products Laboratory, for all the data on chemical analyses of the different preservatives, and to Drs. R. H. True, F. D. Heald, E. P. Meinecke, Caroline Rumbold, and Mr. W. H. Long, of the Bureau of Plant Industry, for many helpful criticisms of the manuscript. The investigations were conducted at the Forest-Products Laboratory, Madison, Wis., maintained by the Forest Service of the United States Department of Agriculture in cooperation with the University of Wisconsin.

NOTE.—This bulletin gives the results of a series of investigations conducted at the Forest-Products Laboratory, Madison, Wis., as to the preservative value of various oils and salts and their toxic effect on wood-destroying fungi.

economic considerations as far as the preservatives used are concerned. These preservatives have in a general way proved to be fairly good, but recently further efforts have been made to supplement them, or perhaps to substitute for them under certain conditions other substances which may be more applicable to certain requirements.

In addition to the toxicity of a preservative toward wood-destroying organisms, its value to the trade will depend largely upon its varied physical and chemical properties, as well as upon certain economic considerations involved. The present publication, however, deals only with the toxic properties. Considerable literature on the toxic effect upon plant and animal life of various chemical substances, both inorganic and organic, has accumulated, but relatively little work has been done with wood-destroying fungi, and any attempt to draw analogies would be misleading.

Even among fungi the toxic concentration of a given preservative will vary, depending upon the organism, the concentration of the preservative, and the growth conditions, such as the composition of media and the temperature. In order to call attention to the fundamental character of certain of these variations and to illustrate the points involved, a brief survey of some of the work of other investigators is here presented before the results of our own work are discussed. No attempt has been made to review all the literature on this extensive topic, and only a few references which serve to illustrate the points the writers desire to emphasize are included.

HISTORICAL.

STIMULATION BY TOXIC SUBSTANCES.

Many investigators have established the fact that certain substances that are poisonous in higher concentration exert only a stimulating effect in extreme dilution. For many years the fluorin compounds have been known to have this stimulating effect upon fermentation. Ono (22)[1] and Raulin (24) have found similar favorable influences exerted by lithium nitrate and sodium fluorid upon algæ and by mercuric chlorid and copper sulphate upon fungi. Fred (9), in his studies on nitrogen fixation, denitrification, ammonification, and putrefactive processes in soils, due to certain bacteria and yeasts, concludes that the yield is in proportion to the toxic stimuli. Likewise, Clark (3, p. 400), in his work on the toxic effect of many acids and salts upon fungi, found that many deleterious agents which at certain concentrations retard germination or early growth afterwards cause a great acceleration of mycelial development.

[1] Figures in parentheses refer to the bibliography at the end of this bulletin.

VARIATION IN TOXICITY OF CHEMICAL SUBSTANCES TO DIFFERENT FORMS OF PLANT LIFE.

A review of the literature on the action of various toxic agents shows that the different forms of plant and animal life often behave very differently toward the same chemical substance. However, on account of the complexity of the digestive and absorptive processes in the higher animals, particularly man, a direct comparison of these forms with plant life is of little value, although the economic consideration of safety in the handling of substances in commercial use is of great importance.

A few general statements to indicate in a concrete form the differences in behavior between the larger plant groups, as well as individual species, will illustrate the point which it is desired to make.

It is unfortunate that the work of different authors can not, in many instances, be directly compared, on account of differences in the method employed. However, much of value can be deduced from the few available examples.

In his valuable work, Clark (3) calls clearly to the attention the variations among different species of molds. Certain toxic agents are shown to present great differences in this respect and others only slight ones. Even the stage of development of a single organism is of great importance, the conidia of the five species used proving more sensitive than the mycelium, so that the inhibition point for spore germination can not safely be considered as the toxic point for the development of mycelium.

Other species of fungi, however, may behave differently from the ones Clark worked with, for Rumbold (25, p. 431) has recently shown that the ascospores and conidia of the blue-stain fungus (*Ceratostomella* sp.) are more resistant than the mycelium to sodium hydroxid and sodium carbonate.

Another interesting phase of the question has been studied by Pulst (23). This investigator shows that the common green mold (*Penicillium glaucum*) has the power of gradually increasing its resistance to toxic agents. He claims that the individual itself without change of generation, but after a somewhat longer period of time, works up its resistance to copper sulphate to a high degree. He also shows by experiment that spores sown from generation to generation on progressively increasing concentrations of this salt likewise attain greatly increased resistance. Similarly, this newly developed resistance is evidenced by an increased rate of growth. For instance, when spores produced on a 3.2 per cent solution of copper sulphate are transferred to new media of like concentration, the mold will fruit again in about 10 days, while spores obtained from a culture containing no toxic substance and transferred in exactly the same manner require more than three months to reach the same stage of development.

Seed plants.—A great amount of work has been done to ascertain the effect of various toxic substances on the roots of higher plants. A discussion of this work, however, is not essential to the present paper beyond showing that a considerable difference exists between the behavior of this group of plants as compared with the lower forms.

In the comparison by Harvey (11) of his own work on an alga (*Chlamydomonas multifilis*) with that of True and Hunkel (30) on a flowering plant (*Lupinus albus*), both investigators using the ortho, meta, and para compounds of dihydric phenol, cresol, and phthalic acid, the alga was found to withstand a concentration three to eight times as high as the flowering plant.

Another striking illustration of this varied response to the same toxic solution is recorded by Heald (12, p. 130), who found a fungus vigorously growing on pea roots which had been killed by hydrochloric acid. The average death point for five species of molds studied by Clark (3, p. 306) was $\frac{n}{3.3}$ HCl (1.1 per cent), while the three species of flowering plants investigated by Heald (12, p. 132) succumbed at $\frac{n}{1600}$ HCl or less.

When copper sulphate was used, Kahlenberg and True (14) found that 0.00062 per cent was sufficient to kill the roots of *Lupinus albus*.

After many experiments, Clark (3, p. 396) concludes that in the case of mineral acids a concentration of 2 to 400 times the strength fatal to the higher plants is required to inhibit the germination of mold spores under favorable conditions.

Bacteria.—Although no direct comparison of bactericidal and fungicidal action is available, the experiments being usually performed under somewhat different cultural conditions, the work of McClintic (17) on zinc chlorid indicates a high resistance of certain bacterial organisms. This investigator found that a 5 per cent solution of zinc chlorid applied for one hour was not sufficient to kill *Bacillus communis*, while a 25 per cent solution required 10 minutes to cause death. At this latter concentration 30 minutes was required to kill another bacterial organism (*Staphylococcus pyogenes aureus*).

Spores of bacteria are well known to be very resistant to various agents. In the case of *Bacillus subtilis*, they are reported to have survived a 50 per cent solution of zinc chlorid for 40 days.

These figures are of interest when one recalls that a 3 to 6 per cent solution of this salt is the usual concentration employed in the preservation of wood.

Yeasts.—Yeasts seem to behave toward many salts and acids very differently from seed plants and fungi. Bokorny (1) has re-

cently reported the effect of about 50 different salts and acids upon yeasts, as compared with other organisms, and has found them generally to be more resistant than algæ or flowering plants. Silver nitrate, which is very deadly to many molds, bacteria, and algæ (the bacterium *Staphylococcus pyogenes* requiring only 0.0002 per cent to check growth; the algæ, Spirogyra and Cladophora, only 0.0001 per cent), will not kill yeast until the concentration reaches 0.001 per cent. Similarly, mercuric chlorid is toxic to Spirogyra in a 0.000001 per cent solution, but a 0.01 per cent solution is required to kill beer yeast.

Molds.—The common molds, such as Penicillium, Aspergillus, Sterigmatocystis, and others, taken as a whole, are highly resistant to toxic agents as compared with the true wood-destroying fungi.

Whereas much experimental work has been done on the former, comparatively little has been carried out on the latter group.

The so-called *Penicillium glaucum* Link., which in the light of recent work has been shown to consist of a group of several distinct species of Penicillium, to which the composite name was indiscriminately applied, is one of the most resistant molds recorded. Pulst (23), Clark (3), and Guéguen (10) all agree that from 16 to 21 per cent of copper sulphate is required to stop its growth, and Pulst claims that it will even germinate and fruit in a 33 per cent solution of this salt or a 38 per cent solution of zinc sulphate if allowed to develop a sufficiently long time, i. e., from three to five months.

Clark (3) has tested the effect of some 28 salts and acids upon four or five of the common molds, the tests being made in hanging drop cultures of beet infusion. His table of toxicities indicates that such salts as mercuric chlorid, potassium bichromate, silver nitrate, and potassium chromate are approximately 400 times as effective against these organisms as copper sulphate, sulphuric acid, hydrochloric acid, and zinc sulphate, the comparison being based on molecular solutions.

EFFECT OF COMPOSITION OF MEDIUM ON TOXICITY.

The toxicity of a substance may vary for the same organism when culture media of different compositions are employed. This is due, in large part, to the chemical or physical affinity of some substances for certain constituents of the media, or possibly to some change in the permeability of the plant protoplasm. The well-known reaction of some copper salts with sugars and of mercuric chlorid with albuminous compounds, or the effect of adsorption will serve to illustrate the point.

The most careful work on toxicity has been conducted, using pure distilled water as a medium. However, the use of this method with fungi is practically limited to the germination of spores; nutrient

substances must be added if further growth is desired, and the addition of each nutrient substance introduces a new factor of error.

Unlike bacteria, which can be grown well in synthetic liquid media of known composition, wood-destroying fungi prefer a more complex and solid medium for their satisfactory development. This latter, as a rule, consists of a mixture of meat broth and sugars solidified by agar-agar or gelatin.

Various investigators have used different types of media and different methods, and this accounts in large part for the variability in results. Some, as Clark (3), have used simple plant decoctions, others bouillon, and still others a nutrient agar or gelatin modified in various ways as to available carbon and nitrogen.

Le Renard (16), in his work on *Penicillium crustaceum*, shows that toxicity is closely associated with the composition of the medium and in the same medium varies somewhat with its concentration.

Likewise the presence or absence of certain constituents may determine the temperature which an organism will endure on different media, for Thiele (27) has shown that the maximum temperature for the growth of *Penicillium glaucum* on grape sugar is 31° C.; on salts of formic acid, 35° C.; and on glycerin, 36° C.

Hoffmann (13) states that in the case of *Merulius lachrymans* a slight growth takes place even at 30° C. on certain liquid media, while on solid media (5 per cent agar-agar) the fungus was killed at that temperature. He likewise thinks that as a fungus becomes accustomed to a certain culture medium in its development it gradually overcomes certain unfavorable conditions.

So far as the writers are aware at the present time, the media most satisfactory for the growth of wood-destroying fungi are not free from the objection of being complex, variable, and more or less unknown in their chemical composition; however, certain synthetic media are being developed in the course of the work which show promise of being satisfactory. In an effort, however, to secure results comparable as far as possible with those of certain European investigators, such as Malenković and various workers at Munich, and also Rumbold in this country, the malt-extract agar medium used by these workers has been adopted. This medium will be described later.

EFFECT OF ADSORPTION ON TOXICITY.

The apparent diluting effect which inert, practically insoluble matter exerts on toxic substances has been often observed. For instance, the injurious effect of poisons is not so noticeable when seedling roots are placed in sand and watered with toxic solutions of definite concentration as when grown directly in such solutions. This phenomenon of the removal from solution of a part of the toxic substance by nearly insoluble material, such as glass, quartz, pottery,

hemp and cotton fibers, and starch grains, comes under the general term "adsorption." It is often explained as a direct physical affinity of the toxic chemical for the inert substance; that is, a condensation of the substance on the surface or in the interstices of the insoluble matter, or the formation of a solid solution of the two, but chemists and physicists are not at all in agreement in regard to these explanations.

Among others, True and Gies (29) and True and Oglevee (31) worked upon this problem, using seedlings of *Lupinus albus* and a number of inorganic and organic compounds. As adsorbing agents such substances as sand, glass, filter paper, and paraffin were applied. With copper sulphate they found that at least twice the usual toxic concentration could be endured by the Lupinus roots when a sufficient quantity of the insoluble matter was added to the hypertoxic solution. In summarizing their work they remark:

> It appears in general that the presence of a considerable body of certain insoluble substances in solutions of strongly toxic compounds both organic and inorganic in their nature, be they electrolytes or not, tends to decrease the toxic activity of the solutions in question. On the whole this ameliorating action is more clearly marked in case the poisonous solutions concerned are dilute solutions of strong poisons than when relatively concentrated solutions of weaker poisons are concerned.

Fitch (8) conducted a series of experiments with sulphuric acid and copper sulphate, using pottery, glass, sand, and filter paper as the adsorbing agents and two common molds (*Aspergillus niger* and *Penicillium glaucum*) as the test organisms. She established for fungi the same phenomena of dilution that a number of other workers had found to hold for flowering plants.

The diversity of results secured when toxic substances are tested on various media, particularly such as contain starch grains and similar materials in suspension, no doubt is explained, in part at least, on the basis of adsorption.

RELATION OF TEMPERATURE TO FUNGOUS GROWTH AND TOXICITY.

It is well known that temperature exerts a vital influence on the growth and development of fungi. Not alone is the temperature range which permits the growth of the organisms highly variable, but also the optimum temperature in many cases varies for the different species. Thus, for nine species of wood-destroying fungi studied, Falck (5, 6) indicated a growth range lying between 3° and 44° C., with the corresponding optima between 18° and 35° C. For *Merulius domesticus* (= *M. lachrymans* in part) this optimum falls between 18° and 22° C.[1]; for *Coniophora cerebella*, 22° to 26° C.; for *Polyporus vaporarius spumarius*, 26° C.; for *Lenzites abietina*, 29.5° C.; for

[1] Hoffmann (13) states that under certain conditions of culture this optimum may be raised so as to fall between 18° and 26° C.

Lenzites sepiaria, 28° to 32° C.; and for *Lenzites thermophila*, 35° C. Below these temperatures growth becomes greatly lessened as the minimum is approached, while a rise of 4 to 8 degrees above the optimum often causes a total inhibition of growth or even death in the case of very sensitive species.

Different stages of the same fungus may also offer a different resistance to temperature changes, this being much less under moist than under dry conditions. For instance, Falck (7, p. 339) found that fresh fruit bodies of *Merulius domesticus* were killed in 30 minutes at 40° to 42° C., and in 15 minutes at 46° C., while from 12 to 16 hours were required to kill dry spores at 42°.

As compared with this fungus, the same author shows that agar cultures of *Lenzites sepiaria* can survive more than three hours at 60° C.

The resistance of a fungus to toxic substances is greatest under temperature conditions most favorable to its development. After conducting a series of tests on several molds to determine the germinative capacity of the spores in varying concentrations of nitric and sulphuric acids and copper sulphate at different temperatures, either directly in the solutions or after removal to nutrient media following immersion for 24 hours, Brooks (2) states that "in most cases the deleterious action increased very rapidly with rise in temperature," but that "in all instances the injurious effects were least at the optimum for the fungus."

RELATION OF LIGHT TO FUNGOUS GROWTH.

Light also exerts an appreciable effect on the development of wood-destroying fungi. This is evidenced in two ways: (1) By its influence on the growth of the mycelium, and (2) by the rôle it plays in the production of normal fruiting bodies. In most instances at least, partial illumination is essential to normal fruiting. The effect on the rate of growth of the mycelium, however, is less marked, but still quite appreciable. Of seven species of wood-destroying fungi studied, Hoffmann (13) reports that growth in the dark was from 4.1 to 17.8 per cent (average 9.9 per cent) better than in sunlight. In carrying his experiments still further and examining the effect of the red and blue ends of the spectrum, respectively, he found that the former gave 2.6 per cent better growth in the case of *Paxillus acheruntius* and 59.3 per cent in the case of *Polyporus vaporarius* (average for nine fungi 14.6 per cent).

WHAT DETERMINES TOXICITY?

An adequate discussion of the subject of what determines toxicity would lead us into one of the most difficult fields of biological chemistry and physiology, hence, for the purposes of this publication, the writers omit reference to a great mass of literature covering the

more involved aspects of the question and merely bring forward a few of the points which serve to illustrate certain phases.

In the previous discussion it has been seen that the degree of toxicity manifested is relative and closely associated with the environmental conditions and the particular physiological constitution of the individual organisms under consideration, as well as with the concentration of the different toxic substances employed and the chemical and physical relations which these bear to the media upon which the organisms are grown. Why certain concentrations of substances are toxic to one plant and not to another, or why the same species varies in its tolerance to a certain toxic agent, is more or less obscure. According to Heald (12, p. 126) it may be a case of "adaptation and adjustment," and this is at least suggested by the work of Pulst (23) in increasing the resistance of *Penicillium glaucum* to copper sulphate. In support of his view Heald further states:

> Those substances which are poisonous to plants are generally such substances as are not accessible to plants in their normal habitats, at least to any extent, while those substances which are generally present in the soil have no injurious effect, or at least not in the same degree of concentration at which we find them in the soil.

However, for the purposes of the present paper the question of how the toxic substances exert their effect is not so near to the point as is the question of what particular components of the substances are the effective ones. On the basis of the separation of compounds into their constituent ions (elements or radicals) when brought into solution, many efforts have been made by comparison of different substances which have certain ions present in varying proportions to determine the most active part of the molecule. As many substances, particularly the more complex, do not become completely dissociated in solution, experimental work largely draws its inferences from the simpler compounds, mainly the inorganic.

As a result of work on such ionized molecular solutions, investigators quite generally agree that in case of the salts of heavy metals, like copper and mercury, it is the metallic ion that is largely effective. In the case of strong acids, such as hydrochloric and sulphuric, the hydrogen ion is said to be the principal toxic element. The work of Kahlenberg and True (14) proves the great activity of hydrogen and shows that in mixtures of such acids the toxicity is proportional to the number of free hydrogen ions present.

In 1900 True (28) published an account of the investigation of 20 acids, both inorganic and organic, together with their sodium salts, in an effort to extend our knowledge of the effective toxic elements, the toxicity tests being conducted on the roots of *Lupinus albus*. With the simple inorganic acids, which readily dissociate in solution, he corroborated earlier views that the H ion gives the greater part of the toxicity to the solution, the corresponding sodium salts of the

acids being only slightly toxic. With the organic acids, in which ionic dissociation is usually less complete, he also found that the relative importance of the H ions in general varied with the percentage of dissociation. If the dissociation was relatively slight, the nonionized molecule itself exerted the predominating influence. In general, the anions of organic acids were found to possess relatively slight toxic properties, oftentimes so slight as to be almost negligible, and, since both the sodium ions and the anions were usually but weakly toxic, it followed, as a rule, that sodium salts showed but 0.5 to 3 per cent of the toxic value of the corresponding acids. Carboxyl hydrogen proved much more toxic than hydroxyl hydrogen. Since in the phenols this latter form of combination occurs, and since these substances do not ionize, the toxicity here must be referred entirely to the undissociated molecule.

In order to throw further light on the behavior of phenols and their derivatives True and Hunkel (30) extended their investigations on *Lupinus albus* to this group. The results bear out their earlier conclusions that electrolytic dissociation of phenylic bodies plays but a very subordinate rôle in determining their toxicity. However, in a few instances, such as with picric and salicylic acids, the cresols, and the mononitrophenols, electrolytic dissociation is said to exert a pronounced influence. Some phenols also, like pyrocatechol and hydroquinone, which are comparatively unstable, may quickly change to constituents even more fatal than H ions. Certain radicals seemed also to have specific properties when introduced into the molecule. For instance, the number of hydroxyl groups appeared to have little influence, while the introduction of the methyl group into the benzene nucleus increased the toxicity to a considerable but variable degree, as shown by the cresols and less plainly by orcinol; however, replacing the H of a hydroxyl group by a CH_3 group had little effect. The introduction of the isopropyl group into the cresols further increased their toxicity. The presence of one or more nitro groups likewise increased toxicity to a great degree, but the number of these groups seemed to make little difference.

Similarly, in the case of certain organic compounds (cf. 7, pp. 351–352), Ehrlich and Bechhold have shown that the introduction of halogen and alkyl groups into the benzol ring increases the toxicity of phenols to diphtheria bacteria, two molecules of pentabromphenol being about equal to 40 molecules of trichlorphenol and 100 molecules of phenol. On the other hand, the introduction of the carboxyl group was said to lessen toxicity.

Likewise, Falck (7, pp. 355, 357) states that nitrophenols and dinitrophenols are considerably more toxic than phenol and more so when the nitro groups are in the ortho position than when in the meta or para position. The most effective of 19 nitrophenols which he tested

in Petri dishes on agar against the fungus *Coniophora cerebella* were the sodium or potassium salts of dinitrophenol ($C_6H_3 \cdot (NO_2)_2$[2.4] ONa) and dinitro-orthocresol ($C_6H_2 \cdot CH_3$ [2] $\cdot (NO_2)_2$ONa).

Generalizations, however, are not always applicable by analogy and may serve only for certain limited groups. In his work on numerous fluorin salts Netzsch (21) found that the fluorin ion was the most active, the relative toxicity of the simpler and consequently more readily dissociated salts, at least, being in direct proportion to the amount of fluorin in the molecule. When the acid (HF) itself, however, was under consideration it was found to be even more toxic than its simple metallic salts, indicating the great activity of free hydrogen.

TESTS OF THE TOXICITY OF WOOD PRESERVATIVES.

It is only within the past decade that laboratory tests to determine the relative toxicity of substances adapted particularly to wood preservation have been undertaken. These lack the refinement of earlier work, as it was not the intention to enter into the question as to why and how a substance was toxic, but merely to determine how much of a given poisonous substance was necessary to inhibit the growth of fungi, particularly the wood-rotting forms. The result is that different investigators have used different methods, different culture media, different organisms, temperature conditions which were often not the optimum for the fungi concerned, and ofttimes also impure chemicals and composite oils, such as creosotes, that no other investigator is able to duplicate except from the same sample.

The problem has been attacked in two ways: (1) By mixing the preservative under consideration with various types of culture solutions, usually solidified by the addition of agar-agar or gelatin, and inoculating with the organisms desired; and (2) by injecting the preservative into wood and exposing the blocks thus treated to the action of wood-destroying fungi.

Tests of this sort were first suggested by Malenković (18) in 1904. The results of his work were first published in an Austrian military journal and later (19) amplified and printed in book form. He lays no claim to refinement of work, so it is difficult to correlate his results with later ones, except in a general way. The larger part of his experiments were carried out by injecting the preservative into wood, but a few beaker tests were made according to the following plan:

> Five glass beakers were filled with 100 c. c. of 10 per cent gelatin or 2 per cent agar media, and to each was added a certain amount of the antiseptic, such as 0.5, 1, 1.5, and 2 grams. The media were then melted, thoroughly stirred, and allowed to cool. Then, without any previous sterilization, a trace of some mold (unknown to the experimenter) was transferred to the surface of the media, and the cultures were set away in a dark, damp place. After 14 days observations were made to determine whether the surface had become moldy. The concentration that prevented mold growth was recorded as the toxic point for the preservative in question.

As has been shown in the preceding discussion, tests that are conducted under any other than pure-culture conditions are not directly comparable with each other, for the different organisms react in an entirely different way to the same chemical substance. Moreover, the use of molds which at most produce but slight effect upon wood gives no more than the roughest approximation as to how wood-destroying organisms would behave under similar conditions.

In 1910 Netzsch (21) conducted an exhaustive series of experiments on the toxicity of fluorin compounds. As these compounds have only recently entered into the field of wood preservation, and as many of them have proved to be toxic agents of high efficiency, his work is of great technical value. He carried out the work much as Malenković and other investigators have done, both by mixing the substances in gelatin culture media and injecting them into wood, but his tests on culture media were carried out under sterile conditions in flasks, tests tubes, or Petri dishes, so that many of the objections to the work of Malenković were eliminated. Into the gelatin media were introduced varying proportions of equimolecular solutions of the fluorin compounds. The culture vessels were then inoculated, using both *Coniophora cerebella* (a true wood destroyer) and the green mold, *Penicillium glaucum*, the former being maintained for about four weeks in an incubator at 20° to 21° C. His results, showing the point of inhibition of growth, are presented on the basis of one gram molecule of the preservative to the number of liters of culture media necessary to secure the proper concentration. In the present paper this ratio has been changed to the percentage basis (weight of preservative in volume of media), in order to compare his results with those of other investigators.

About this same time Seidenschnur (26), head chemist of the wood-preservation laboratory of the Rütgerswerke-Aktiengesellschaft, at Berlin (Charlottenburg), presented the results of a few tests upon the comparative toxicity of zinc chlorid and tar oils. His experiments were conducted in test tubes containing gelatin media mixed with varying proportions of the antiseptics. After the mixture was prepared, the tubes were sterilized for one-half hour at 80° C.[1] The tubes were then slanted and inoculated with *Penicillium glaucum*. The toxic point was not determined, but the relative efficiency of the two substances was compared in parallel cultures.

During 1911, J. M. Weiss (33, 34), chemist in the technical laboratories of the Barrett Manufacturing Co., New York City, published the results of a number of experiments to test the relative antiseptic value of creosotes and other oils. The substances were mixed in agar media. The organisms used consisted of a bacterium (*Bacillus subtilis*), a yeast (*Saccharomyces glutinis*), and a species of Penicillium.

[1] This treatment, however, is usually considered insufficient to insure sterility.

An effort was made to handle them in pure culture under sterile conditions. The selection, however, of test organisms which play at most but a slight rôle in the decay of timber is not to be recommended. As many factors of error as possible should be eliminated from such tests, for there are certain to be many remaining after all precautions are taken.

During the same year Rumbold (25) carried out a series of tests with different wood preservatives, using agar media in Petri dishes as well as toasted bread soaked in the antiseptics. In the case of the agar cultures, the media and preservative were mixed before sterilization. This procedure is known to lead to very erroneous results with certain substances, such as zinc chlorid and copper sulphate. In all cases the preservative and media should be sterilized separately and heated no higher than is necessary during the mixing, in order to avoid as far as possible any chemical combination which tends to occur. The higher concentrations of the salts mentioned above cause a liquefaction of agar or gelatin media when sterilized together. One test conducted in our laboratory showed that zinc chlorid at 0.6 per cent concentration when sterilized after mixing allowed even more growth of *Fomes annosus* than 0.2 per cent when the two components were not sterilized together. Concentrations of the sterilized mixture below about 0.4 per cent appeared to be stimulative, giving a white, fluffy growth, which was more luxuriant than in the creamy check cultures and which grew up over the under side of the covers of the Petri dishes.

The use of bread for culture media likewise is objectionable, for the starch therein contained possibly acts as a diluting agent, as already indicated in the discussion of the phenomena of adsorption. For instance, in comparing Rumbold's tests of sodium carbonate on bread and on agar it is seen that considerably more of the preservative is required to check the growth of the organisms when the former medium is used.

In 1912, Falck (7), and Dean and Downs (4), published the results of work on various wood preservatives in agar media, using wood-rotting organisms.

The former covered a wide range of possible preservatives (some 60 or 70), including phenols and cresols and their derivatives, benzol derivatives, fluorin compounds, acids, alkalies, and inorganic metallic salts. The work appears to have been very carefully done and is an extremely valuable contribution to the subject. It is open to the objection, however, that the tests were of too short a duration.

Dean and Downs report only a few tests on tar oils in a bean-agar medium, using the cosmopolitan wood-rotting fungus *Polystictus versicolor*. These investigators introduced a method of preparing creosote emulsions with gum arabic, which was considered advanta-

geous,[1] particularly with heavy oils. They also attempted to improve upon the usual method of inoculating the surface of the culture with the mycelium of the test organism by cutting a small block out of the medium, placing the transferred mycelium in the aperture, and then covering this with the portion of medium which was originally removed. They claimed this would give a more accurate indication of whether the fungus was really growing on the treated medium or only on the fragment of medium which must necessarily accompany the mycelium when it was transferred. This appears, however, from work in our laboratory, to be a refinement of doubtful expediency, for it has often resulted that when fresh, actively growing mycelium is placed in intimate contact with the poison it will be directly killed, while if it has the opportunity to recover its vigor to a certain extent after the disturbance in its growth equilibrium, due to cutting and removal from the original culture, it may eventually withstand concentrations which would otherwise be fatal.

The more important results of these different investigators are presented in Table IV (pp. 31–34).

TESTS CONDUCTED AT THE FOREST-PRODUCTS LABORATORY.

SCOPE OF THE WORK.

The experimental work in wood preservation at the Forest-Products Laboratory includes a physical, chemical, and pathological examination of various substances which may have a possible value in the industry (32). Therefore, since toxicity is but one factor, conclusions regarding the service value of these substances should not be drawn without giving due consideration to other factors.[2] The pathological tests are made in Petri dishes, using agar media, or by injecting the preservatives into wood and exposing the wood to the action of wood-destroying organisms. Only the Petri-dish method is herein described. This method has the advantage of giving results from which at least tentative conclusions can be drawn in a relatively short time. Conversely, it is open to certain objections for which due allowance must be made in generalizations regarding the possible behavior of a preservative when placed under service conditions. However, in experimental work on the toxicity of different chemical substances it is often very necessary to secure indicatory results as soon as possible. In this way many substances may be eliminated which are not worthy of further trial. After a preservative has been shown to possess high toxic properties under

[1] From the purely physical side of preparing the preservatives so they can be more readily handled the gum-arabic emulsions have proved satisfactory to the writers, but the gum arabic apparently reduces the toxicity to such an extent as to forbid its use in comparative tests. This fact has been determined since this manuscript was prepared.

[2] See U. S. Dept. of Agriculture Bulletin 145, "Tests of wood preservatives."

Petri-dish conditions, tests on its properties when injected into wood should follow, under both laboratory and service conditions.

Recently, an attempt has been made by a European investigator (20) to correlate Petri-dish results directly with service values. First, the preservatives were grouped as nearly as possible according to their permanence in wood. Then, knowing the average length of life of the treated timbers, the amount of preservative necessary to inject to give this life, and the toxic point of the substances as indicated by Petri-dish tests, a curve was plotted using the first factor as the axis of ordinates and the ratio existing between the second two as the axis of abscissas. From this curve the investigator would predict the possible service value of any new preservative of like permanence in wood merely from the known Petri-dish ratio by locating the point at which its ordinate intersects the standard curve.

Such mathematical calculations are interesting, but must necessarily be very limited in their application, since such variables as the solubility and volatility of the preservatives, the nature of the timber treated, and the soil and weather conditions to which the treated wood is exposed must necessarily exert a great influence on the length of the life of the material.

At the Forest-Products Laboratory, 2,400 Petri-dish tests have been made to date on 54 different substances; however, not all are sufficiently complete to be reported. These include a few water-soluble salts, but in the main they comprise various oils and tars. These preservatives have been for the most part submitted by American and European cooperators interested in having the substances examined.

The tests were conducted using two wood-destroying organisms, *Fomes annosus* Fr. and *Fomes pinicola* (Sw.) Fr., which have a wide American and European distribution and are very important in the decay of wood, particularly coniferous timber. The former is undoubtedly the most serious fungus of coniferous mine timbers in the United States.

In general, the molds used by other investigators may be considered more resistant than the true wood-destroying fungi, but the writers have considered it advisable to use only wood-destroying forms, in order to eliminate any possibly erroneous inferences.

METHODS OF TESTING TOXICITY.

The method of conducting the tests was in principle the same as that used by other investigators, merely involving the mixing of the various preservatives in definite proportions with media nutrient to fungi. However, an attempt was made to refine the methods as far as possible, so as to eliminate certain sources of error to which attention has already been called.

A culture medium made according to the following formula was used:

Extract of 1 pound lean beef in distilled water	1,000 c.c.
Löfflund's malt extract	25 grams.
Agar-agar	20 grams.

(Carefully filtered, but reaction not adjusted; slightly acid.)

This is the formula largely used by German investigators. It is a good medium for the development of fungi, but, like all other media of organic and often unknown composition, offers the objection of possible chemical reaction with certain preservatives. However, such synthetic media as were experimented with proved very poor substrata for the development of the organisms.

The above medium after melting was measured [1] into 50-c. c. glass bottles with carefully ground glass stoppers, usually 17 c. c. to a bottle, using a standardized 17-c. c. pipette or a 25-c. c. graduate. One check was usually prepared for each series of concentrations and to this was added sufficient distilled water to make 20 c. c. The stoppers were then sealed in with a rubber-glycerin burette-cock grease and capped with a small piece of muslin. The bottles were clamped in specially constructed frames (Pl. I, fig. 1) and given a sterilization of 25, 20, and 20 minutes, respectively, at 100° C. on successive days.

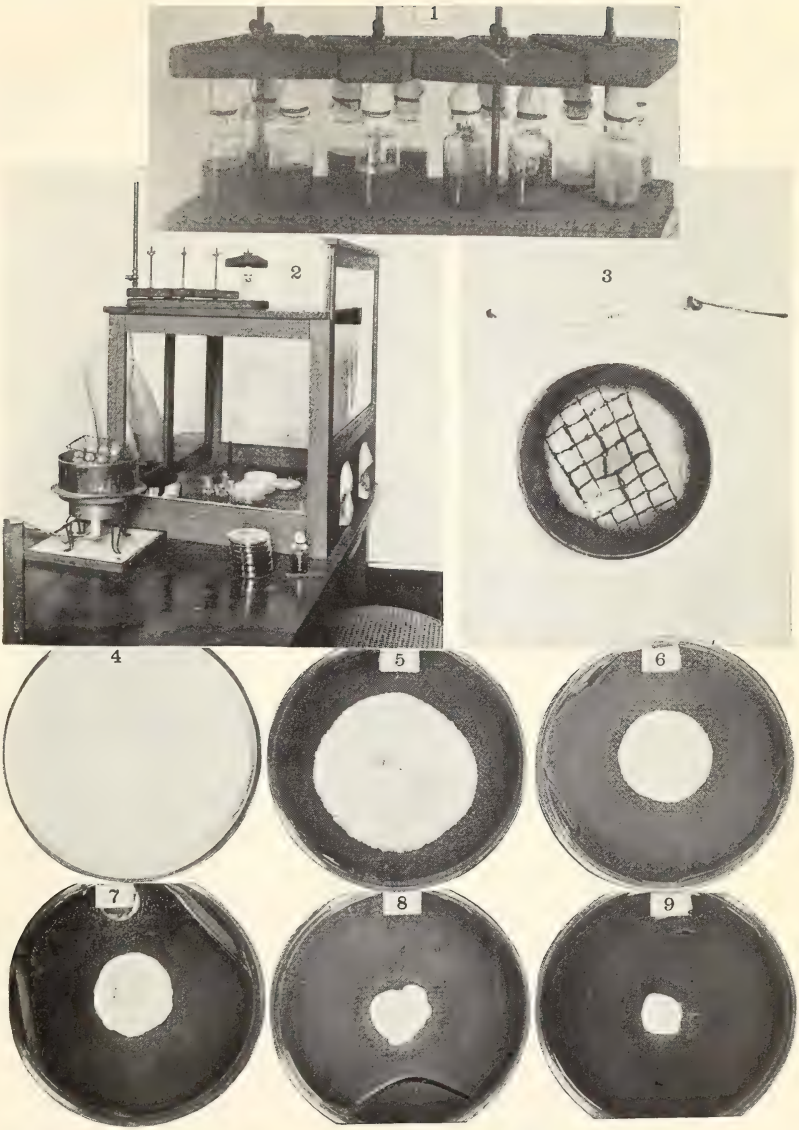
The handling of the preservatives involved slight modifications for individual cases, but in all instances concentrations are based on the actual weight of the preservatives in grams in 20 c. c. agar-preservative mixture.

With inorganic salts soluble in water solutions were prepared varying from 3 to 10 per cent concentration (grams in 100-c. c. solution), and these were used by measuring into 50-c. c. bottles, similar to those used for agar, the desired amount of solution, using either a 10-c. c. or a 25-c. c. standardized burette graduated in twentieths or tenths of a cubic centimeter, respectively. To each bottle was then added sufficient distilled water to make 3 c. c. In all cases concentrations were based on the weight of dry salt present.

All other preservatives were weighed into the 50-c. c. bottles on an analytical balance, and enough distilled water was added to make 3 c. c. In the case of a few viscous oils, namely, coal-tar creosote, coal-tar creosote Fraction V, wood tar, and wood creosote, which do not readily emulsify with water, 5 to 33⅓ per cent stock emulsions [2] were prepared, using equal amounts of gum arabic and preservative and diluting with distilled water to the desired concentration. These emulsions were then used in place of the crude preservatives.

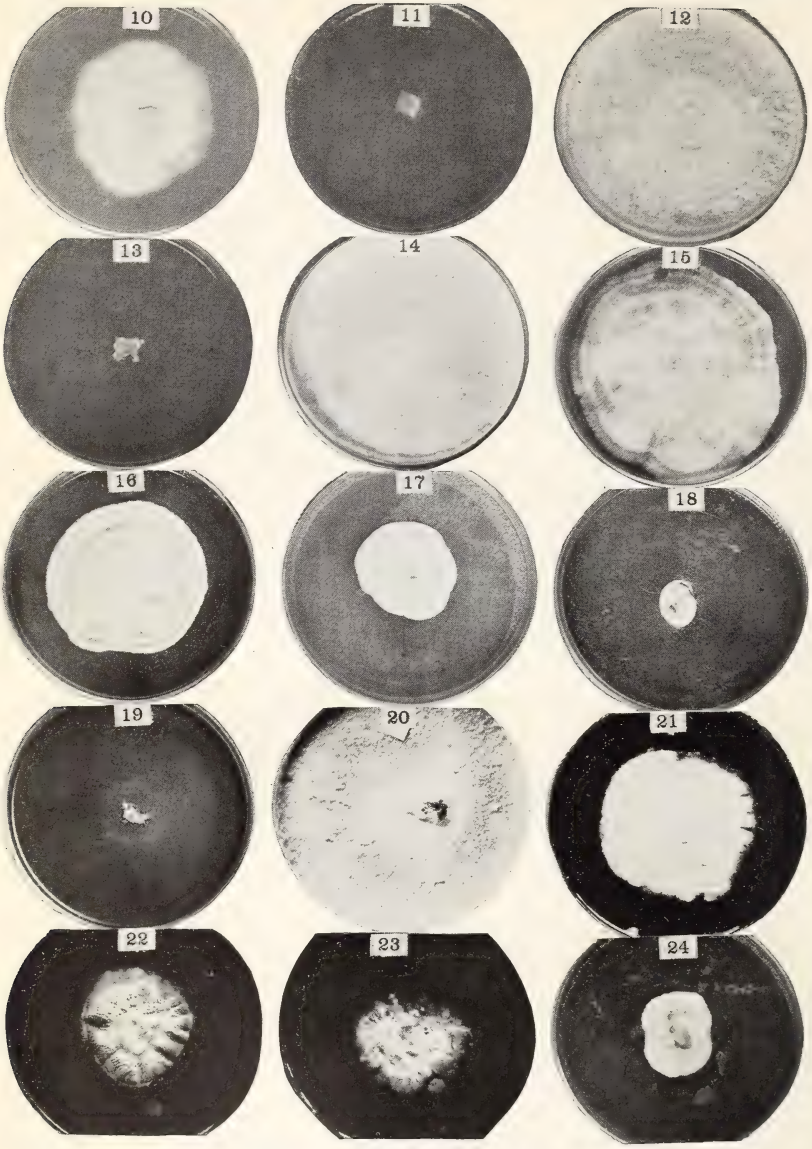
[1] In all measurements of agar one-half c. c. excess was allowed to cover the amount adhering to the glass containers.

[2] This method usually produced a quite permanent emulsion.



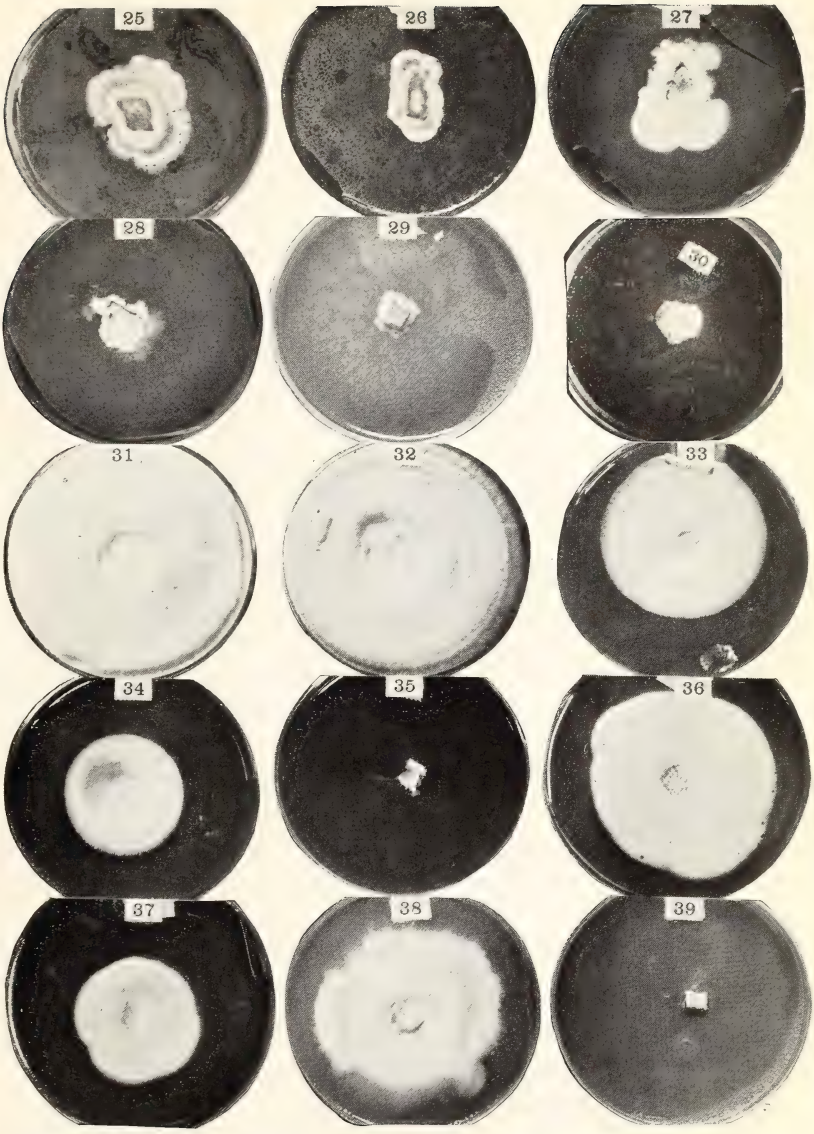
TOXICITY STUDIES: APPARATUS AND PETRI-DISH CULTURES.

FIG. 1.—Frame for agar and preservative bottles during sterilization. FIG. 2.—Inoculation case, showing water bath on hot plate and other apparatus used. FIG. 3.—Petri-dish culture of *Fomes annosus* cut into squares ready for transferring to the test plates; platinum needle with flattened tip used in the operation. FIGS. 4 to 9.—Petri-dish cultures of *Fomes pinicola* on different concentrations of coal-tar creosote, grade C, after 5 weeks: 4, Check; 5, on 0.075 per cent; 6, on 0.1 per cent; 7, on 0.125 per cent; 8, on 0.15 per cent; 9, on 0.175 per cent.



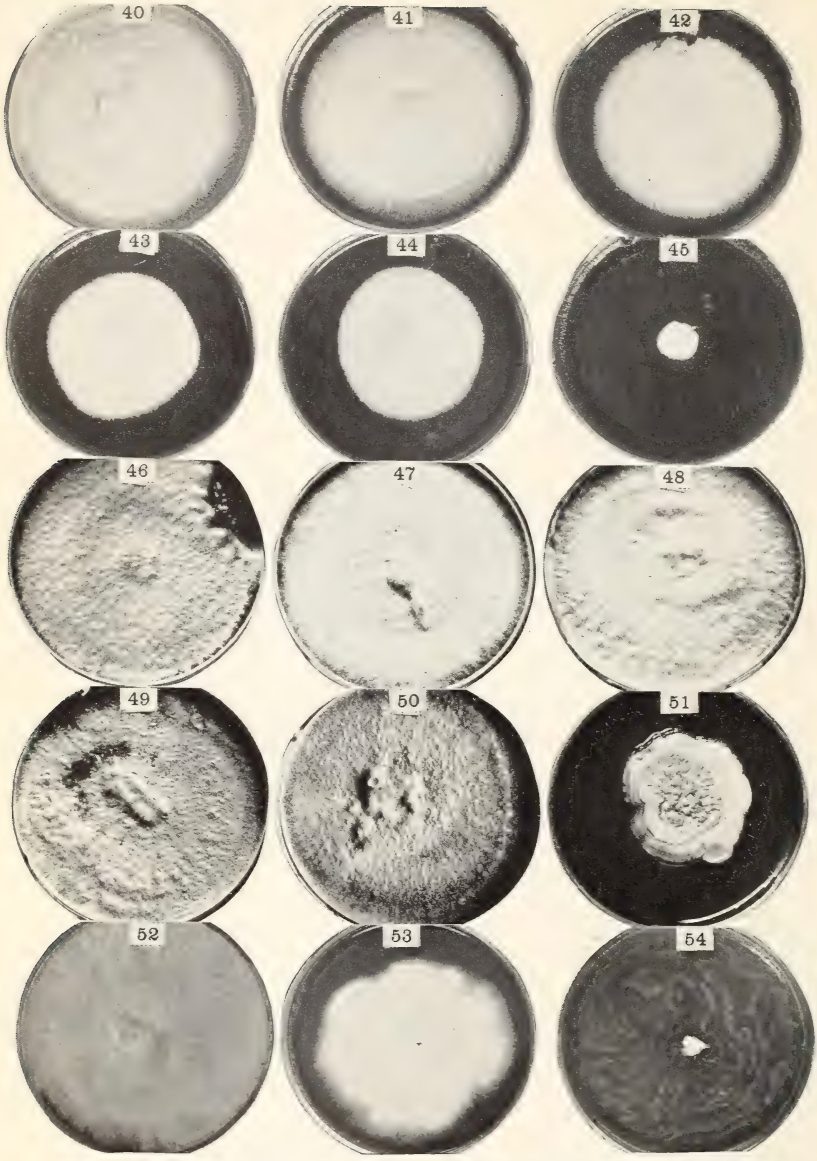
TOXICITY STUDIES: PETRI-DISH CULTURES OF FOMES ANNOSUS AND FOMES PINICOLA.—I.

FIGS. 10 and 11.—*F. annosus* on different concentrations of coal-tar creosote, Fraction I, after 5 weeks: 10, on 0.275 per cent; 11, on 0.3 per cent. FIGS. 12 and 13.—*F. pinicola* on different concentrations of coal-tar creosote, Fraction II, after 5½ weeks: 12, On 0.125 per cent; 13, on 0.15 per cent. FIGS. 14 to 19.—*F. pinicola* on different concentrations of coal tar creosote, Fraction IV, after 9 weeks: 14, Check; 15, on 0.025 per cent; 16, on 0.05 per cent; 17, on 0.075 per cent; 18, on 0.1 per cent; 19, on 0.125 per cent. FIGS. 20 to 23.—*F. annosus* on different concentrations of coal-tar creosote, Fraction V, after 7 weeks: 20, Check; 21, on 0.1 per cent; 22, on 0.5 per cent; 23, on 1 per cent. FIG. 24.—*F. pinicola* on 1 per cent concentration of coal-tar creosote, Fraction V, after 6 weeks.



TOXICITY STUDIES: PETRI-DISH CULTURES OF FOMES ANNOSUS AND FOMES PINICOLA.—II.

FIGS. 25 and 26.—*F. pinicola* on different concentrations of United Gas Improvement Co. 1.07 oil, No. 1101, after about 5 weeks: 25, On 20 per cent; 26, on 40 per cent. FIGS. 27 and 28.—*F. annosus* on different concentrations of water-gas tar distillate (sp. gr. 0.995 at 60° C.), after 6 weeks: 27, On 0.2 per cent; 28, on 0.3 per cent. FIG. 29.—*F. annosus* on 2.2 per cent concentration of S. P. F. carbolineum after about 6 weeks. FIG. 30.—*F. annosus* on 0.15 per cent concentration of coal-tar creosote, Fraction III, after 6 weeks. FIGS. 31 to 35.—*F. pinicola* on different concentrations of wood creosote (Douglas fir) after about 2½ weeks: 31, Check; 32, on 0.05 per cent; 33, on 0.1 per cent; 34, on 0.125 per cent; 35, on 0.15 per cent. FIGS. 36 and 37.—*F. annosus* on different concentrations of wood creosote (Douglas fir) after about 4 weeks: 36, On 0.2 per cent; 37, on 0.4 per cent. FIGS. 38 and 39.—*F. annosus* on different concentrations of cresol calcium after about 6 weeks: 38, On 0.14 per cent; 39, on 0.28 per cent.



TOXICITY STUDIES: PETRI-DISH CULTURES OF FOMES ANNOSUS AND FOMES PINICOLA.—III.

FIGS. 40 to 45.—*F. pinicola* on different concentrations of wood tar (hardwood) after 2 weeks: 40, Check; 41, on 0.2 per cent; 42, on 0.3 per cent; 43, on 0.4 per cent; 44, on 0.5 per cent; 45, on 0.6 per cent. FIGS. 46 to 48.—*F. annosus* on different concentrations of wood tar (hardwood) after about 3 weeks: 46, On 0.1 per cent; 47, on 0.5 per cent; 48, check. FIGS. 49 to 51.—*F. annosus* on different concentrations of copperized oil: 49, On 0.5 per cent after about 3 weeks: 50, on 1.75 per cent after about 3 weeks; 51, on 36 per cent after about 5½ weeks. FIG. 52.—*F. pinicola* on 50 per cent concentration of None-Such Special after about 3½ weeks. FIGS. 53 and 54.—*F. pinicola* on different concentrations of zinc chlorid (commercial) after 4 weeks: 53, On 0.7 per cent; 54, on 0.75 per cent.

In a few instances where the preservatives were low in toxic properties more than the specified 3 c. c. was necessary in order to secure the higher concentrations, and in these cases it became necessary to take into consideration the excess of preservative, and, considering it roughly as having the specific gravity of water, to reduce the agar by just this amount, in order that the combined volume might not exceed 20 c. c.

The concentrations to be used in the first series[1] of experiments on a given preservative were governed largely by the judgment of the investigator. The results thus obtained usually determined between what limits it was necessary to continue the work. The experiments were then carried on between these points, usually to within an accuracy of about 10 per cent for the actual and total inhibition point for each preservative. Thus, if growth stopped at 0.5 per cent or below, the tests were carried to the nearest 0.05 per cent; if between 0.5 and 1 per cent they were carried to the nearest 0.1 per cent, and so on up to the highest concentrations employed, usually 40 per cent, which would thus be tested to the nearest 4 per cent. This 40 per cent concentration is equivalent to an injection of 24.9 pounds of the preservative per cubic foot, and it was thought unnecessary from a practical standpoint to go above this point.

After the proper quantities of preservative had been placed in 50-c. c. glass-stoppered bottles, these were sealed and sterilized in exactly the same way as the agar bottles and along with them.

As a few experimental weighings before and after sterilization indicated that no loss occurred, even of such volatile substances as are contained in the lowest fractions of coal-tar creosote, the method may be considered safe.

After sterilization, both the agar and preservative bottles were heated on the water bath, then transferred to a sterile culture case (Pl. I, fig. 2), where the hot agar was poured into the preservative bottle and thoroughly mixed. In some cases one or two sterile glass beads were added to the preservative bottles to facilitate the mixing. These were removed later.

The agar-preservative mixtures were then poured into sterile Petri dishes 100 mm. in diameter and 10 mm. deep. After cooling, each plate was inoculated at the center with a weft of mycelium 5 or 6 mm. square, cut from a Petri-dish culture (Pl. I, fig. 3) 2 to 3 weeks old of the fungus desired, either *Fomes annosus* Fr. or *Fomes pinicola* (Sw.) Fr. The dishes thus prepared were then placed in an incubator and held at approximately 25° C. for periods varying from 4 to 10 weeks, usually from 4 to 6.

[1] A series consists of a set of progressively increasing concentrations of a given preservative, tested at the same time against the action of a single fungus.

In addition to the possibility, in some instances, of chemical combinations between the preservative and the media, there will also necessarily be a slight change in concentration, due to the drying out of the media when held in Petri dishes for six to eight weeks. Likewise, during this interval of time certain volatile constituents, particularly the lighter oils, may escape from the media.

In recording observations of the behavior of the fungi toward the preservative, rapidity and amplitude of growth, together with any other peculiarities in appearance, were noted, inspection being made about once a week.

One very interesting feature of the tests was the development of a "halo" around either the living or the dead transfers, or in advance of the fungous growth on the check cultures. These halos differed in appearance on the different preservatives, sometimes being lighter, sometimes darker, than the surrounding medium. In order to determine whether the change was due to advance submerged hyphæ, several transfers were made from the halos to fresh sterile agar, but as no living organisms were demonstrated by this test or by microscopical examination to be present it appears to be an advance physicochemical change in the media, arising, perhaps, from the diffusion of enzyms from the transfer, as Kellerman (15) has recently demonstrated for cytase produced in fungus cultures.

DEVELOPMENT OF FOMES ANNOSUS AND FOMES PINICOLA.

IN NONTOXIC CHECK CULTURES.

In the check cultures, *Fomes annosus* produces a rather compact creamy growth (Pl. II, fig. 20, and Pl. IV, fig. 48), forming an abundance of the characteristic conidia described by Brefeld. *F. pinicola* (Pl. I, fig. 4; Pl. III, fig. 31; and Pl. IV, fig. 40), on the other hand, develops a fluffy, deep, white mycelium of considerably more rapid growth than *F. annosus*. At 25° C., *F. pinicola* develops a radial growth of 24 mm. in 9 days (average of 14 tests) and covers the plate in 15 days (15 tests); *F. annosus* develops 15 mm. in $8\frac{2}{3}$ days (19 tests) and covers the plate in $20\frac{1}{2}$ days (12 tests).

IN CULTURES CONTAINING TOXIC SUBSTANCES.

The rate of growth of each of these two fungi on toxic media is usually considerably retarded, in many cases strongly so, as compared with check cultures. In some instances where very low concentrations of certain substances are used, a stimulating effect is observed, but this condition is reversed with increased concentrations. The stimulated growth on zinc-chlorid concentrations when heated in the presence of the culture media (such concentrations often being far above those necessary to kill when not so heated together) is

readily explained on the basis of chemical combination with the media.

The length of time required to make an initial growth on different toxic media varies from a very few days up to seven weeks or more; check cultures usually start within two or three days. Table I illustrates this for a few preservatives.

TABLE I.—*Time required by Fomes annosus on 0.2 per cent concentrations and F. pinicola on 0.1 per cent concentrations to make initial growth from mycelium at 25° C.*

Preservative used.	F. annosus.	F. pinicola.
	Days.	*Days.*
Check cultures	2 to 3	2 to 3
Sodium fluorid	20	24
Coal-tar creosote	18	21
Coal-tar creosote, Fraction II	15	18
Coal-tar creosote, Fraction III	40	23
Coal-tar creosote, Fraction IV	.....	53

It is thus seen that no growth demonstrable to the naked eye on any of the concentrations mentioned occurred within a period of two weeks, and the error which would occur in discontinuing the tests at the end of 8 to 10 days, as several investigators have done, becomes very evident.

RECORD OF TESTS CONDUCTED.

A description of each of the 18 preservatives used, accompanied by individual tables and notes showing the rate, amplitude, and appearance of growth on the different concentrations, as compared with the check cultures, is given on the following pages. Under the heading "Concentration of the preservative" in each table the average radial distance to which the fungus has grown from the margin of the transfer at the end of the test is indicated by numerals,[1] the position of the first zero marking the killing point: Thus, 4 = 30 to 40 mm.; 3 = 20 to 30 mm.; 2 = 10 to 20 mm.; 1 = 1 to 10mm.; 0 = no growth.

Sodium fluorid.

[Laboratory sample No. 1929. Purchased from Eimer & Amend, Chicago, Ill.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).					
	Number.	Duration.		0.05	0.1	0.15	0.2	0.25	Check.
		Weeks.							
Fomes annosus	9	4	1	4	3	2	1	0	4
F. pinicola	11	5 to 7	1	3	2	0	.....	.....	4

[1] It should be kept in mind that the growth rate designated as "4" is not necessarily the same on different preservatives or different concentrations of the same preservative. All cultures which have produced a growth of at least 30 to 40 mm. radius at the end of the test are included. Some of these may have reached this point in two to three weeks, as in the case of the checks, while others may have required the full test period; hence, the data as presented show only the relative retarding effect of the higher concentrations.

Fomes annosus: In one week, the radial growth of the check was 15 mm.; 0.05 per cent, 20 mm.; 0.1 per cent, 15 mm.; 0.15 per cent, 3 mm. In four weeks concentrations up to 0.15 per cent showed 20 to 30 mm.; 0.2 per cent, about 2 mm. Thus, the rate of growth on 0.05 and 0.1 per cent concentrations equaled or exceeded that of the checks, but the higher concentrations produced a decided inhibition. The growth on the toxic media appeared very much as on the check.

Fomes pinicola: In two weeks, the radial growth of the check was 40 mm.; 0.05 per cent, 9 mm.; 0.1 per cent, 4 mm.; no growth on 0.15 per cent. After four to six weeks, no growth on 0.15 per cent. The growth on the toxic media was fluffy, white, and quite similar in appearance to that on the check.

Zinc chlorid.

(Pl. IV, figs. 53 and 54.)

[Laboratory sample No. 2239. Cooperator, Grasselli Chemical Co., Cleveland, Ohio. Commercial salt, meeting the specifications of the American Railway Engineering and Maintenance-of-Way Association.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).											
	Number.	Duration.		0.1	0.2	0.3	0.4	0.45	0.475	0.5	0.6	0.65	0.7	0.75	Check.
		Weeks.													
Fomes annosus	33	5 to 8	1	4	1	1	1	1	1	0	----	------	------	------	4
F. pinicola	33	4 to 8	2	----	----	----	----	----	------	----	4	4	4	0	4

Fomes annosus: In 18 days, the radial growth of the checks reached about 35 mm.; 0.05 per cent, about equal to check; 0.15 per cent, 8 mm.; 0.3 per cent, 1 mm. There was usually no growth on higher concentrations until after about four weeks, and it was comparatively slight even after six to eight weeks, reaching only 1 to 8 mm. The toxic cultures generally were denser and of brighter tan color than the check.

Fomes pinicola: In two weeks, the radial growth of the check reached 40 mm.; 0.6 and 0.7 per cent, 10 mm. After four weeks, 0.6 and 0.7 per cent reached 35 mm. On the lower concentrations the growth was more fluffy than that on the check.

Sapwood antiseptic.

[Laboratory sample No. 1611. Cooperator, J. M. Long, Chicago, Ill. Formula (by weight, in water solution): NaCl, 2.92 per cent; $CaSO_4$, 0.246 per cent; $ZnSO_4+7\ H_2O$, 0.246 per cent; $CuSO_4+5\ H_2O$, 0.182 per cent; $FeSO_4+4\ H_2O$, 0.0605 per cent.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).						
	Number.	Duration.		16⅔	25	30	50	60	75	Check.
		Weeks.								
Fomes annosus	17	4 to 5	.....	4	4	3	2	2	1	4

Fomes annosus: In about three weeks the radial growth of check reached 40 mm.; 50 per cent, 10 to 15 mm.; 75 per cent, very slight growth. Penicillium developed abundantly on 100 per cent. The preservative up to 25 per cent concentration showed a decided stimulating effect.

The first concentration is based on the solution, as given above, which was prepared and submitted by the cooperator. Above 16⅔ per cent it was necessary to use a more concentrated solution, and a strength six times the original formula was prepared.

Coal-tar creosote, grade C.

(Pl. I, figs. 5 to 9.)

[Laboratory sample No. 1074. Purchased from the Creosote Supply Co., Chalmette, La. Liquid at room temperature, 8.4 per cent water; specific gravity, 1.0483 at 60° C.; flash point, 93° C.; burning point, 100° C.; 11 per cent distills below 205° C.; 54.1 per cent distills below 275° C.; 74.1 per cent distills below 320° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).											
	Number.	Duration.		0.075	0.125	0.175	0.2	0.225	0.3	0.35	0.4	0.45	0.5	0.551	Check.
		Weeks.													
Fomes annosus	44	4 to 8	1	....	....	....	2	1	1	1	1	1	1	0	4
F. pinicola	21	4 to 6	1	3	2	1	1	0	....	....	....	....	....	....	4

[1] These values are based on gum-arabic emulsions. Later work, using the pure preservative, indicates that the toxic point may fall somewhat lower.

Fomes annosus: In 17 days the radial growth of the check reached about 40 mm.; 0.2 and 0.275 per cent showed initial growth only. In three to four weeks, 0.2 per cent showed 10 mm.; 0.25 to 0.5 per cent, 2 to 3 mm. In six weeks, 0.525 per cent showed only initial growth. In seven weeks, 0.5 and 0.525 per cent reached 2 to 9 mm. The growth on the toxic media was about the same in appearance as on the check.

Fomes pinicola: In two weeks the radial growth of the check reached about 40 mm.; 0.075 per cent, 3 mm.; no growth above this point. In four weeks, 0.075 per cent showed 25 mm.; 0.1 per cent, 13 mm.; 0.15 per cent, 6 mm. After five weeks, 0.175 and 0.2 per cent reached 3 mm.; above this point all growth was inhibited. The growth on the toxic media was dull white, with a crinkled edge.

Coal-tar creosote, Fraction I.

(Pl. II, figs. 10 and 11.)

[Laboratory sample No. 1094. Cooperator, Semet-Solvay Co., Ensley, Ala. Light liquid at room temperature; specific gravity, 0.934 at 60° C.; flash point, 62° C.; burning point, 69° C.; 78.3 per cent distills below 215° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).					
	Number.	Duration.		0.2	0.225	0.25	0.275	0.3	Check.
		Weeks.							
Fomes annosus	33	4 to 6	3	4	4	2	1	0	4
F. pinicola	13	4 to 6	1	4	0	.....	.....	.....	4

Fomes annosus: In 10 to 14 days the radial growth of the check reached 20 mm.; 0.2 to 0.25 per cent, from 1 to 4 mm. In four weeks, 0.2 to 0.225 per cent reached 30 mm.; 0.25 to 0.275 per cent, from 7 to 10 mm.; no growth at 0.3 per cent. The toxic cultures produced a thin, stringy growth.

Fomes pinicola: In two weeks the radial growth of the check reached 40 mm.; 0.2 per cent, 1 mm. In four weeks, 0.2 per cent reached 40 mm.; no growth above this point. The toxic cultures produced a fluffy white growth, similar in appearance to that on the check.

Coal-tar creosote, Fraction II.

(Pl. II, figs. 12 and 13.)

[Laboratory sample No. 1106. Cooperator, Semet-Solvay Co., Ensley, Ala. Naphthalene odor; nearly solid at room temperature; specific gravity, 1.003 at 60° C.; flash point, 79° C.; burning point, 85° C.; 9 per cent distills below 205° C.; 95.9 per cent distills below 287° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).						
	Number.	Duration.		0.1	0.125	0.15	0.175	0.2	0.225	Check.
		Weeks.								
Fomes annosus	20	5 to 9	1	.....	3	2	1	1	0	4
F. pinicola	18	4 to 5	2	4	4	0	.....	.....	.....	4

Fomes annosus: In two weeks the radial growth of the check reached 20 mm.; no growth on 0.15 per cent and above. In four weeks, no growth on 0.225 per cent. In six weeks, 0.15 per cent reached 12 mm.; 0.2 per cent, 6 mm.; no growth on 0.225 per cent. The growth on the toxic media was compact and creamy in color.

Fomes pinicola: In two weeks the radial growth of the check reached 40 mm.; no growth on 0.125 per cent. In four weeks 0.1 and 0.125 per cent reached 40 mm.; no growth above this point. The growth on the toxic media was a dingy white and less fluffy than that on the check.

Coal-tar creosote, Fraction III.

(Pl. III, fig. 30.)

[Laboratory sample No. 1107. Cooperator, Semet-Solvay Co., Ensley, Ala. Liquid at room temperature; specific gravity, 1.045 at 60° C.; flash point, 103° C.; burning point, 110° C.; 0.9 per cent distills below 215° C.; 4.7 per cent distills below 275° C.; fraction from 170° to 245° C. more or less solid with naphthalene.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).									
	Number.	Duration.		0.05	0.075	0.1	0.125	0.15	0.2	0.25	0.3	0.325[1]	Check.
		Weeks.											
Fomes annosus	60	4 to 12	2	.....	.....	.....	.....	1	1	1	1	0	4
F. pinicola	14	4 to 7	1	3	2	1	0	.....	.....	.....	.....	.....	4

[1] The radial growth of *Fomes annosus* on concentrations above 0.2 per cent is very slight, usually not exceeding 1 mm., and the actual toxic point is difficult to locate. It lies between 0.25 and 0.35 per cent.

Fomes annosus: In 19 days the radial growth of the check reached 40 mm.; 0.15 and 0.175 per cent, 1 or 2 mm. In five to six weeks 0.3 per cent showed 1 mm. and lower concentrations 1 to 5 mm. The growth on the toxic media was creamy and slightly lighter than that on the check.

Fomes pinicola: In two weeks the radial growth of the check reached 40 mm. In about one month 0.05 per cent showed 3 mm.; 0.075 per cent, 2 mm. In about seven weeks 0.1 per cent showed initial growth; lower concentrations from 16 to 23 mm. The growth on the toxic media was a fluffy white on the lower concentrations; denser on 0.1 per cent.

Coal-tar creosote, Fraction IV.

(Pl. II, figs. 14 to 19.)

[Laboratory sample No. 1108. Cooperator, Semet-Solvay Co., Ensley, Ala. Liquid at room temperature; specific gravity, 1.088 at 60° C.; flash point, 130° C.; burning point, 136° C.; 0.9 per cent distills below 245° C.; 54.3 per cent distills below 320° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).											
	Number.	Duration.		0.025	0.05	0.075	0.1	0.125	0.2	0.9	1	2	3	3.3[1]	Check.
		Weeks.													
Fomes annosus	75	5 to 12	1	....	....	....	....	....	2	2	1	1	1	0	4
F. pinicola	23	5 to 8	1	3	2	2	1	0	....	....	....	....	....	....	4

[1] The growth of *Fomes annosus* on concentrations above 2 per cent is very slight, usually not exceeding 1 mm. in six weeks, and the actual toxic point is difficult to locate. It lies between 2.5 and 3.5 per cent.

Fomes annosus: In 12 days, the radial growth of the check reached 25 mm.; very slight growth on 0.2 to 0.5 per cent. In 18 days, 0.8 to 3 per cent reached 1 to 4 mm. In four weeks, 1.25 to 3 per cent reached 1 to 5 mm. The growth on the toxic media was usually darker than on the check.

Fomes pinicola: In two weeks, the radial growth of the check reached 40 mm.; no growth on 0.025 per cent. In four weeks, 0.025 per cent showed 2 mm.; no growth on higher concentrations. In eight weeks, 0.025 per cent reached 25 mm.; 0.05 per cent, 15 mm.; 0.075 per cent, 10 mm.; 0.1 per cent, 2 mm.; no growth above this point. The growth on the toxic cultures was dull, compact, and crinkled.

Coal-tar creosote, Fraction V.

(Pl. II, figs. 21 to 24.)

[Laboratory sample No. 1109. Cooperator, Semet-Solvay Co., Ensley, Ala. Heavy, tarry liquid; specific gravity, 1.150 at 60° C.; flash point, 172° C.; burning point, 178° C.; 10.1 per cent distills below 320° C.; 63.3 per cent distills below 380° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).								
	Number.	Duration.		0.1	0.5	1	7	7.8	8	30	33[1]	Check.
		Weeks.										
Fomes annosus	42	4 to 9	0	4	3	2	2	.....	1	1	0	4
F. pinicola	30	4 to 8	0	.....	.....	2	1	0	.....	.....	.....	4

[1] These values are based on gum-arabic emulsions.

Fomes annosus: In 10 to 14 days, the radial growth of the check reached about 40 mm.; 5.5 to 30 per cent showed 1 to 4 mm. In four weeks, 5.5 to 7.5 per cent reached 8 to 15 mm.; 15 and 25 per cent, 2 mm.; no growth on 20 per cent gum-arabic emulsion. The growth on the toxic media was dense and creamy on the lower concentrations; submerged at first on the higher concentrations.

Fomes pinicola: In two weeks, the radial growth of the check reached 40 mm.; in three weeks, 1 to 2 per cent showed initial growth; in four weeks, 3 to 6.5 per cent showed first growth; after eight weeks, 4.5 to 5 per cent reached 10 to 20 mm. The growth on the lower concentrations of the toxic media was white and fluffy; on the higher concentrations it was tawny and radiate, with a crinkled edge.

Avenarius carbolineum.

[Laboratory sample No. 1843. Cooperator, Carbolineum Wood Preserving Co., Milwaukee, Wis. Thick liquid at room temperature: specific gravity, 1.126 at 16.5° C.; flash point, 139° C.; burning point, 166° C.; 1.1 per cent distills below 215° C.; 6.1 per cent distills below 275° C.; 1.91 per cent tar acids.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).							
	Number.	Duration.		0.175	0.275	0.3	0.8	1	4	5	5.25	Check.
		Weeks.										
Fomes annosus	53	4 to 10	1	.....	.....	2	2	1	1	1	0	4
F. pinicola	18	5 to 6	2	1	1	0	----	----	----	----	-----	4

Fomes annosus: In 18 days, the radial growth of the check reached nearly 40 mm.; 0.3 per cent, 17 mm.; higher concentrations up to 4 per cent showed 1 to 6 mm. In four to six weeks, 5 per cent reached 1 mm. The growth on the toxic media was similar in appearance to that on the check.

Fomes pinicola: In two weeks the radial growth of the check reached 40 mm.; 0.175 per cent showed no growth. In four weeks, 0.175 to 0.275 per cent reached 1 mm. The growth on the toxic media was compact and darker than that on the check.

S. P. F. carbolineum.

(Pl. III, fig. 29.)

[Laboratory sample No. 1844. Cooperator, Bruno-Grosche & Co., New York, N. Y. Thick brown liquid, specific gravity, 1.127 at 16° C.; flash point, 133° C.; burning point, 157° C.; less than 9 per cent distills below 245° C.; about 30 per cent distills below 320° C.; 2.42 per cent tar acids.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).							
	Number.	Duration.		1	1.5	1.75	2	3	4	4.5[1]	Check.
		Weeks.									
Fomes annosus	73	5 to 8	2	1	1	1	1	1	1	0	4

[1] The growth of *Fomes annosus* on concentrations above 3 per cent is very slight, usually not exceeding 1 mm. in six weeks, and the actual toxic point is difficult to locate. It lies between 4 and 5 per cent.

Fomes annosus: In four weeks the radial growth of the check reached about 40 mm.; 1 and 1.5 per cent, 1 mm. In six weeks, 1 and 1.5 per cent showed 5 mm.; 1.75 to 4 per cent, 1 to 5 mm. Tests were not made on concentrations below 1 per cent. The growth on the toxic media was quite similar in appearance to that on the check.

Water-gas tar distillate.

(Pl. III, figs. 27 and 28.)

[Laboratory sample No. 2235. Cooperator, United Gas Improvement Co., Philadelphia, Pa. Greenish brown liquid; specific gravity, 0.995 at 60° C.; flash point, 81° C.; burning point, 93° C.; 3.3 per cent distills below 180° C.; 61.7 per cent distills below 275° C.; 80.3 per cent distills below 320° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).								
	Number.	Duration.		0.1	0.2	0.3	0.35	0.4	0.45	0.5	0.6	0.65	Check.
		Weeks.											
Fomes annosus	58	5 to 8	0	4	3	2	2	1	1	1	1	0	4

Fomes annosus: In 12 days the radial growth of the check reached 15 mm.; 0.1 per cent, 2 mm. In three weeks, 0.1 per cent showed about 20 mm.; 0.2 per cent, 5 mm. In six weeks, 0.2 per cent reached 17 mm.; 0.25 per cent, 15 mm.; 0.3 per cent, 5 mm.; 0.35 to 0.6 per cent, from 1 to 12 mm. The growth on the toxic media was of a brighter creamy appearance than that on the check.

United Gas Improvement Co. 1.07 oil (water-gas tar distillate).

(Pl. III, figs. 25 and 26.)

[Laboratory sample No. 1101. Cooperator, United Gas Improvement Co., Philadelphia, Pa. Mobile, oily liquid, with kerosene odor; specific gravity, 1.058 at 60° C.; flash point, 48° C.; burning point, 65° C.; 9.04 per cent distills below 205° C.; 24.24 per cent distills below 315° C.; 67.9 per cent distills below 378° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).										
	Number.	Duration.		0.1	3	3.5	5	6	6.5	10	25	30	40	Check.
		Weeks.												
Fomes annosus	31	4 to 8	.....	4	4	3	2	.....	.....	2	1	1	1	4
F. pinicola	22	4 to 5	.....	.....	2	2	2	2	1	.....	1	.....	1	4

Fomes annosus: In 10 to 14 days, the radial growth of the check reached 28 mm.; 40 and 50 per cent, 2 mm. In four to six weeks, 40 and 50 per cent showed nearly 10 mm. The growth on the toxic media appeared denser and of a brighter tan color than that on the check.

Fomes pinicola: In two weeks, the radial growth of the check reached almost 40 mm.; 3 to 29 per cent, 2 to 7 mm. In four to five weeks all concentrations between 3 and 40 per cent showed 2 to 15 mm. The growth on the toxic media appeared velvety, compact, and creamy, while that on the check was a fluffy white.

Wood tar (hardwood).

(Pl. IV, figs. 41–47.)

[Laboratory sample No. 1561. Cooperator, Marden, Orth & Hastings, Chicago, Ill. Black, viscous liquid, with pyroligneous odor; specific gravity, 1.195 at 60° C.; flash point, 90° C.; contains 24 per cent water; 11.7 per cent distills below 105° C.; 50.9 per cent distills below 244° C.; decomposition occurs above 230°.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).							
	Number.	Duration.		0.2	0.5	0.7	0.75	0.9	1	1.25[1]	Check.
		Weeks.									
Fomes annosus	50	4 to 6	0	.....	4	.....	4	4	2	0	4
F. pinicola	30	4 to 5	1	4	4	4	0	.....	.....	.....	4

[1] Based on gum-arabic emulsions.

Fomes annosus: In 9 to 10 days, the radial growth of the check reached 12 mm.; 0.5 to 0.8 per cent, 1 to 2 mm. In two to four weeks the check reached 40 mm. and concentrations up to 1 per cent showed 10 to 35 mm. The growth on the toxic media was dark creamy and somewhat denser than that on the check.

Fomes pinicola: In two weeks the radial growth of the check reached 40 mm.; 0.5 per cent, 10 mm.; no growth above this. In four to five weeks 0.2 to 0.7 per cent showed 40 mm.; 0.725 per cent, 10 mm.; no growth on higher concentrations. The growth on the toxic media was luxuriant, white, and fluffy.

Wood creosote (Douglas fir).

(Pl. III, figs. 32–37.)

[Laboratory sample No. 1099. Cooperator, Logged-Off Land Utilization Co., Seattle, Wash. Black liquid, with a strong pyroligneous odor; specific gravity, 1.052 at 60° C.; flash point, 45° C.; burning point, 85° C.; contains 8.35 per cent water; 7.55 per cent distills below 100° C.; 54.69 per cent distills below 245° C.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).									
	Number.	Duration.		0.1	0.125	0.15	0.2	0.25	0.4	0.45	0.6	0.65[1]	Check.
		Weeks.											
Fomes annosus	55	4 to 7	0	4	.....	3	3	2	2	1	1	0	4
F. pinicola	26	4 to 6	0	4	4	4	0	.....	.....	.....	.....	.....	4

[1] These values are based on gum-arabic emulsions.

Fomes annosus: In 11 days the radial growth of the check reached 25 mm.; 0.15 to 0.30 per cent reached 3 to 6 mm. In 15 days, 0.1 per cent showed 30 mm. In four weeks, 0.15 to 0.30 per cent reached 18 to 28 mm. Usually, from four to six weeks were required for initial growth on 0.6 to 0.625 per cent. The growth on the toxic media was dense, dark creamy, occasionally zonate.

Fomes pinicola: In eight days the radial growth of the check reached 15 mm.; 0.025 to 0.075 per cent, 11 to 20 mm. In three weeks, 0.025 to 0.1 per cent covered the plates; 0.125 per cent reached 17 mm.; 0.15 per cent 4 mm. In 34 days the initial growth appeared on 0.175 per cent. The growth on the toxic media up to 0.125 per cent was white and luxuriant, exceeding that on the check.

Cresol calcium.

(Pl. III, figs. 38 and 39.)

[Laboratory sample No. 2086. Cooperator, Blagden, Waugh & Co., London, England.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).		
	Number.	Duration.		0.14	0.28	Check.
		Weeks.				
Fomes annosus	8	4 to 7	1	4	0	4

Fomes annosus: In about two weeks the radial growth of the check reached 20 mm.; 0.14 per cent, 3 mm. After three weeks the check showed 30 mm.; 0.14 per cent, 11 mm. After six weeks, 0.14 per cent reached 30 mm.; no growth on 0.28 per cent. The growth on the toxic media occurred in alternating light and dark zones.

A diversion from the usual method of weighing the preservative was necessary with this substance, since on sterilizing at 100° C. the preservative would form a hard crust on the sides of the bottles. To obviate this difficulty, the average weight of a drop from a small pipette was obtained (four tests). This was found to be 28 milligrams, the drops varying not over 2 or 3 milligrams. The preservative was then added directly to the media bottles in quantities of one, two, three, or four drops, the killing point lying between one and two drops, which corresponds to 0.14 and 0.28 per cent, respectively.

Copperized oil.

(Pl. IV, figs. 49 to 51.)

[Laboratory sample No. 1095. Cooperator, Ellis-Foster Co., New York, N. Y. Probably a crude petroleum containing a slight amount of copper and sufficient vegetable oil to form a homogeneous solution; specific gravity, 0.937 at 25° C.; flash point, 125° C.; burning point, 164° C.; less than 0.2 per cent distills below 215° C.; 30.2 per cent distills below 320° C.; about 80 per cent distills below 360° C.; copper content, 0.34 per cent.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).							
	Number.	Duration.		3	5	15	30	33	36	40	Check.
		Weeks.									
Fomes annosus	40	4 to 9	1	4	4	3	2	2	2	0	4
F. pinicola	25	4 to 6	1	4	4	4	....	....	....	4	4

Fomes annosus: In 18 to 22 days the radial growth of the check reached 40 mm.; the toxic concentrations up to 6.25 per cent showed the same growth, except that the check was denser. In about three weeks 15 to 30 per cent reached 1 to 11 mm. In nine weeks, 30, 33, and 36 per cent showed 12 to 20 mm. On the less toxic media alternating zones of lighter superficial growth and darker submerged growth occurred. On the high concentrations either a black submerged growth or a light-brown superficial growth appeared.

Fomes pinicola: In two weeks the radial growth of the check reached 40 mm.; 15 to 26 per cent, 20 to 27 mm.; 40 per cent, 2 mm. In six weeks all concentrations up to 40 per cent reached about 40 mm. The growth on the toxic media was fluffy, but darker than that on the check.

None-Such Special.

(Pl. IV, fig. 52.)

[Laboratory sample No. 2696. Cooperator, George M. Saums Co., Trenton, N. J. Yellow, oily liquid with strong varnish or paint odor; claimed by the manufacturers to waterproof and give a hard finish to timber, as well as prevent or stop decay; chemical composition unknown to us.]

Fungus.	Tests.		Times killing point verified.	Concentration of the preservative (per cent).								
	Number.	Duration.		1	5	15	25	30	35	40	45	Check.
		Weeks.										
Fomes annosus	28	4	.....	4	4	4	4	4	4	4	4	4
F. pinicola	29	3 to 4	.....	4	4	4	4	4	4	4	....	4

Fomes annosus: In 10 to 14 days the radial growth of the check reached 20 mm.; concentrations from 5 to 45 per cent showed approximately 40 mm. The preservative appears to exert a nutritive or stimulative effect, rather than any toxic action, although the fungus will not grow on the pure preservative. The growth on the treated media is dark and submerged, rising to the surface later to form a very dense creamy mycelium.

Fomes pinicola: In eight days the radial growth of the check reached about 30 mm.; 0.25 to 3 per cent, 15 to 20 mm. In two to three weeks 35 to 50 per cent showed 20 to 40 mm. The growth on all concentrations up to 50 per cent almost equaled that on the check.

DISCUSSION OF TESTS.

Table II gives the results of investigations in our laboratory in the use of such preservatives as have been sufficiently checked to permit statements regarding their toxicity to the fungi noted under

the test conditions outlined. In this table the preservatives are arranged in the order of their toxicity, beginning with the most toxic.

TABLE II.—*Killing point of Fomes annosus and F. pinicola for the various preservatives, compared with coal-tar creosote, No. 1074, and zinc chlorid, No. 2239.*

[Results marked with an asterisk (*) were not checked in duplicate, but they are approximately correct.]

Fungus and preservative.	Killing point.		Ratio to creosote.	Ratio to zinc chlorid.
	Per cent.	Pounds per cubic foot.		
Fomes annosus (creosote killing point, 0.55 per cent; zinc-chlorid killing point, 0.5 per cent):				
Coal-tar creosote, Fraction II	0.225	0.140	2.5	2.2
Sodium fluorid	.25	.156	2.2	2
Cresol calcium [1]	0.14–.28	0.087–.175	3.9–1.9	3.6–1.8
Coal-tar creosote, Fraction I	.30	.187	1.8	1.7
Coal-tar creosote, Fraction III	*.325	.203	1.7	1.5
Zinc chlorid	.50	.312	1.1	1
Coal-tar creosote, grade C	.55	.343	1	.91
Water-gas tar distillate No. 2235 (specific gravity 0.995)	*.65	.405	.84	.76
Wood creosote (Douglas fir)	*.65	.405	.84	.76
Wood tar (hardwood)	*1.25	.78	.44	.40
Coal-tar creosote, Fraction IV	*3.30	2.06	.16	.15
S. P. F. carbolineum	4.5	2.8	.12	.11
Avenarius carbolineum	5.25	3.27	.104	.095
Coal-tar creosote, Fraction V	*33	20.59	.017	.015
Copperized oil	40	25	.014	.013
United Gas Improvement Co. 1.07 oil, No. 1101	40+	25+	.014–	.013–
None-Such Special	40+	25+	.014–	.012–
Sapwood antiseptic	75+	.....	.007–	.007
Fomes pinicola (creosote killing point, 0.225 per cent; zinc-chlorid killing point, 0.75 per cent):				
Coal-tar creosote, Fraction III	.125	.078	1.8	6
Coal-tar creosote, Fraction IV	*.125	.078	1.8	6
Coal-tar creosote, Fraction II	.15	.094	1.5	5
Sodium fluorid	.15	.094	1.5	5
Wood creosote (Douglas fir)	*.20	.125	1.12	3.8
Coal-tar creosote, Fraction I	.225	.140	1	3.3
Coal-tar creosote, grade C	.225	.140	1	3.3
Avenarius carbolineum	.30	.187	.75	2.5
Zinc chlorid	.75	.468	.30	1
Wood tar (hardwood)	.75	.468	.30	1
Coal-tar creosote, Fraction V	*7.8	4.87	.029	.096
Copperized oil	40+	25+	.0056–	.019–
United Gas Improvement Co. 1.07 oil, No. 1101	40+	25+	.0056–	.019–
None-Such Special	50+	31.2+	.0045–	.0015–

[1] Killing point lies between the limits given (1 and 2 drops of the preservative in 20 c. c. of the medium).

Table II shows that of the 18 preservatives tested against *Fomes annosus* 6 totally inhibit growth at or below 0.5 per cent, 5 between 0.5 and 3.5 per cent, 1 at 4.5 per cent, 1 at 5.25 per cent, and the remaining 5 show extremely low toxic properties, requiring from 33 to 75 per cent.

Sodium fluorid is particularly toxic, being slightly more than twice as effective as zinc chlorid.

Cresol calcium in these tests shows a high toxicity, and the poor results reported against it in service tests [1] are apparently due to a change in chemical constitution or to leaching, which did not take place under our method of testing.

[1] Unpublished report, Forest-Products Laboratory.

The three lower boiling fractions of coal-tar creosote are highly toxic, exceeding the creosote itself. Fraction IV is only about one-sixth as toxic as creosote. Fraction V, consisting of the last heavy residues, of which only 10 per cent distills below 320° C., is extremely low in toxicity, with a killing concentration of about 33 per cent.

Wood creosote, derived from the destructive distillation of Douglas fir, compares very favorably with coal-tar creosote, notwithstanding its water content of over 8 per cent.

Hardwood tar shows moderate antiseptic properties, proving about one-half as toxic as the softwood creosote.

The two carbolineums are much less toxic than the creosotes tested.

Water-gas tar distillate of low specific gravity appears to be slightly less toxic than coal-tar creosote, while the heavier distillate, represented by United Gas Improvement Co. 1.07 oil, is so low in toxic properties as to appear to be of little value in wood preservation.

The secret product None-Such Special appears to be more nutrient than antiseptic to fungi, so far as these tests indicate; however, the physical properties of the substance when injected into wood may be such as to exclude fungous growth and thus to substantiate the claims made for it. Durability tests on treated wood are highly desired.

Zinc chlorid has a killing point almost identical with coal-tar creosote.

Table II also shows that of 14 preservatives tested against *Fomes pinicola* 8 totally inhibit growth below 0.5 per cent, 2 between 0.5 and 1 per cent, 1 at about 7.8 per cent, and the remaining 3 require over 40 per cent.

Sodium fluorid and coal-tar creosote Fractions II, III, and IV are all extremely toxic to this fungus, the killing points being almost identical.

Coal-tar creosote Fraction I and wood creosote are about three-fourths as toxic as the above; Avenarius carbolineum is about one-half as toxic.

Zinc chlorid in the *Fomes pinicola* list stands only tenth in efficiency, whereas in the *F. annosus* list it stands in fifth place.

The last four preservatives show very low antiseptic properties toward *Fomes pinicola*, as they did toward *F. annosus*.

By comparing the behavior of the two fungi toward the same chemical substances a marked difference will be observed. With the exception of zinc chlorid and copperized oil, *Fomes annosus* is a far more resistant organism than *F. pinicola*, the ratio running as high as 26 to 1 in the case of Fraction IV of coal-tar creosote.

It has often been noted during the course of the experiments that *Fomes annosus*, after a considerable lapse of time, can accommodate itself to rather high concentrations of certain preservatives. Its

normal growth is also relatively slow as compared with that of the other organism. Sometimes on high concentrations of toxic media the fungus would remain dormant from four to seven weeks and then begin a slow development. For this reason it has been difficult in many cases to find the exact inhibition point of a preservative without carrying out a large number of long-continued tests.

On the other hand, *Fomes pinicola* seems rather sensitive to slight changes in concentration, and one can usually judge within a month whether the fungus will develop.

The difference in behavior between these two organisms shows how very necessary it is to make only qualified statements when discussing the relative toxicity of preservatives toward fungi. We have at present no satisfactory way of predicting, except by trial, how a given preservative will react on different organisms.

A direct comparison of toxicities, as given in Table II, shows that in many cases essentially the same order holds, but there are several exceptions, Fractions I and IV of coal-tar creosote and zinc chlorid being the most conspicuous.

In Table III the oils used are grouped according to their nature, in order to show a direct comparison between wood tar, coal tar, water-gas tar, and petroleum products.

TABLE III.—*List of wood-preserving oils tested, showing relation between their specific gravities, boiling points, and toxic properties.*

[Results marked with an asterisk (*) are approximately correct.]

Preservative.	Specific gravity.[1]	Percentage distilling below—							Killing point (per cent).	
		180° C.	215° C.	245° C.	275° C.	305° C.	320° C.	360° C.	Fomes annosus.	Fomes pinicola.
Wood tar (hardwood)	1.195	*27	*31	51	.....	.....	.....	.....	*1.25	0.75
Wood creosote (Douglas fir)	1.052	*16	*31	54.7	.....	.....	.....	.....	*.65	*.20
United Gas Improvement Co. 1.07 oil, No. 1101	1.058	*7	*10	.....	16.3	*22	*27	56.4	40+	40+
Water-gas tar distillate No. 2235	.995	3.3	12.8	37.7	61.7	75.3	80.3	.....	*.65	.....
Coal-tar creosote:										
Grade C	1.048	4.8	17.8	44.4	54.1	67.2	74.1	.....	.55	.225
Fraction I	.934	35.1	78.3	.....	.....	.....	.....	.....	.30	.225
Fraction II	1.003	*2-3	30	*80	*92	.....	.....	.....	.....	.....
Fraction III	1.045	.....	.9	16.2	49.2	77.7	85	.....	*.325	.125
Fraction IV	1.088	.....	.....	.9	4.7	38.5	54.3	.....	*3.30	*.125
Fraction V	1.150	.....	.....	.....	.....	4.1	10.1	48.7	*33	*7.8
Avenarius carbolineum (sp. gr. at 16.5° C.)	1.126	.....	1.1	2.6	6.1	16.4	*29	.....	5.25	.30
S. P. F. carbolineum (sp. gr. at 16° C.)	1.127	.....	.....	*9	.....	.....	*30	.....	4.5	.....
Copperized oil (sp. gr. at 25° C.)	.937	.....	*.2	*3	*10	*22	30.2	*80	40	40+

[1] At 60° C. except as stated for the last three preservatives.

It is interesting to note that the wood-tar and low-boiling water-gas tar and coal-tar distillates tested show very similar toxic properties, while the carbolineums, which consist in the main of the high-boiling constituents of coal-tar creosote, in all cases proved much less toxic to the fungi used.

The toxicity of water-gas tar products is highly variable, much more so than commercial coal-tar products. By decreasing the specific gravity the toxicity rapidly increased. The writers do not wish it to be inferred, however, that this necessarily means that water-gas tar and coal-tar products will prove equally efficient under service conditions. The present results are merely suggestive.

The fractionization of coal-tar creosote gives some interesting data. In the case of *Fomes annosus* the three lower fractions proved considerably more toxic than the creosote itself. In the case of *F. pinicola* the four lower fractions were included. In the former case Fraction II gave the best results and in the latter the greater toxicity fell to Fractions III and IV. This indicates that the middle fractions are the most efficient, but to what group of substances the greater toxicity is due we are not yet prepared to state. The work of other investigators with naphthalene, which is one of the principal constituents of Fraction II, would seem at least to militate against this substance.

The high-boiling carbolineums, which approach Fraction IV in their physical and chemical properties, likewise approach it in their toxic properties.

While the higher boiling constituents proved to be less toxic than the lower boiling ones, their greater permanence in wood under service conditions may at least partially offset the lessened toxic efficiency.

The poor showing made by copperized oil against both fungi indicates that adding small amounts of copper in this form to low-toxic petroleum or vegetable oils will produce a mixture of doubtful fungicidal value.

TOXICITY TO FUNGI OF CERTAIN OF THE MORE IMPORTANT PRESERVATIVES.

In order to bring together in convenient form for comparison the results secured by various investigators in the use of certain important preservative substances, as well as those originating in our own laboratory upon the preservatives mentioned, Table IV has been prepared, indicating the salient features of such tests.

In making comparisons, the sources of error as well as the degree of refinement which the figures represent, should be fully considered.

TABLE IV.—*Toxicity of various preservatives to certain wood-destroying and other fungi.*

Toxic substance.	Organism.	Toxic point.	Culture medium.	Duration of test.	Investigator.
A.—INORGANIC COMPOUNDS.					
		Per cent.			
Ammonium chromate $[(NH_4)_2CrO_4]$.	Coniophora cerebella.	Under 1.....	Agar....	8 to 10 days.	Falck.
Ammonium fluorid $[NH_4F]$, neutral.	.....do.....	Under 0.1...	...do.....	.....do.....	Do.

TABLE IV.—*Toxicity of various preservatives to certain wood-destroying and other fungi*—Continued.

Toxic substance.	Organism.	Toxic point.	Culture medium.	Duration of test.	Investigator.
A.—INORGANIC COMPOUNDS—con.		*Per cent.*			
Ammonium fluorid	Coniophora cerebella	0.123	Gelatin	4 weeks	Netzsch.
Copper fluorid [CuF_2+2H_2O], pure.	do	Under 0.1	Agar	8 to 10 days	Falck.
Copper silico-fluorid [$CuSiF_6$], pure.	do	Under 0.05	do	do	Do.
Copper sulphate [$CuSO_4+5H_2O$].	do	Under 1	do	do	Do.
Copper sulphate	Molds	3 to 5	Gelatin	14 days	Malenkovič.
Ferric fluorid [FeF_3]	Coniophora cerebella	0.132	do	4 weeks	Netzsch.
Ferric sulphate [$FeSO_4+7H_2O$].	do	Under 2	Agar	8 to 10 days	Falck.
Ferrous fluorid [FeF_2]	do	0.155	Gelatin	4 weeks	Netzsch.
Hydrofluoric acid [HF], 100 per cent.	do	Under 0.01	Agar	8 to 10 days	Falck.
Hydrofluoric acid	do	0.05	Gelatin	4 weeks	Netzsch.
Hydrofluorsilicic acid [H_2SiF_6].	do	Under 0.05	Agar	8 to 10 days	Falck.
Hydrofluorsilicic acid	do	0.120	Gelatin	4 weeks	Netzsch.
Magnesium silicofluorid [$MgSiF_6+6H_2O$], 95 per cent.	do	Under 0.067	Agar	8 to 10 days	Falck.
Magnesium sulphate [$MgSO_4+7H_2O$].	do	Over 16	do	do	Do.
Mercuric chlorid [$HgCl_2$]	do	Under 0.1	do	do	Do.
Potassium fluorid [KF], pure.	do	Under 0.05	do	do	Do.
Potassium fluorid	do	0.192	Gelatin	4 weeks	Netzsch.
Sodium chlorid [NaCl]	do	Under 10	Agar	8 to 10 days	Falck.
Sodium chlorid	Mold	Over 5	Gelatin	14 days	Malenkovič.
Sodium fluorid [NaF]	do	2	do	do	Do.
Sodium fluorid, tech. refined.	Coniophora cerebella	Under 0.1	Agar	8 to 10 days	Falck.
Sodium fluorid	do	0.139	Gelatin	4 weeks	Netzsch.
Sodium fluorid, tech	Fomes annosus	0.25	Agar	do	Humphrey and Fleming.
Do	F. pinicola	0.15	do	5 to 7 weeks	Do.
Sodium carbonate [Na_2CO_3].	Coniophora cerebella	Under 0.125	do	10 days	Rumbold.
Sodium silico-fluorid [Na_2SiF_6], about 100 per cent.	do	Under 0.1	do	8 to 10 days	Falck.
Sodium silico-fluorid	do	0.208	Gelatin	4 weeks	Netzsch.
Zinc chlorid [$ZnCl_2$]	do	Under 0.5	Agar	8 to 10 days	Falck.
Zinc chlorid	do	Between 1 and 2.	do	8 days	Rumbold.
Do	Lenzites sepiaria	do	do	do	Do.
Do	Polystictus hirsutus	do	do	do	Do.
Do	P. versicolor	Over 2	do	do	Do.
Zinc chlorid, commercial	Fomes pinicola	0.75	do	4 to 8 weeks	Humphrey and Fleming.
Do	F. annosus	0.5	do	5 to 7 weeks	Do.
Zinc fluorid [ZnF_2]	Coniophora cerebella	0.186	Gelatin	4 weeks	Netzsch.
Acid zinc fluorid, tech	do	Under 0.1	Agar	8 to 10 days	Falck.
Acid zinc fluorid [ZnF_2-HF].	do	0.130	Gelatin	4 weeks	Netzsch.
Zinc silico-fluorid	do	0.159	do	do	Do.
Zinc silico-fluorid [$ZnSiF_6+6H_2O$].	do	Under 0.1	Agar	8 to 10 days	Falck.
Zinc sulphate [$ZnSO_4+7H_2O$].	do	Under 1	do	do	Do.
B.—ORGANIC COMPOUNDS.					
(*a*) *Benzol and phenol derivatives.*					
Anilin [$C_6H_5NH_2$]	Coniophora cerebella	Under 1	Agar	8 to 10 days	Falck.
Cresol [$C_6H_4CH_3OH$]	do	Under 0.125	do	8 days	Rumbold.
Cresol	Lenzites sepiaria	do	do	do	Do.
Do	Polystictus versicolor	do	do	do	Do.
Do	P. hirsutus	Under 0.25	do	do	Do.
Cresol, pure	Penicillium	Under 0.05	do	4 weeks	J. M. Weiss.
Cresol calcium	Coniophora cerebella	Between 1 and 2.	do	8 days	Rumbold.
Do	Lenzites sepiaria	Over 2	do	do	Do.
Do	Polystictus versicolor	Between 1 and 2.	do	do	Do.

TABLE IV.—*Toxicity of various preservatives to certain wood-destroying and other fungi*—Continued.

Toxic substance.	Organism.	Toxic point.	Culture medium.	Duration of test.	Investigator.
B.—ORGANIC COMPOUNDS—continued.					
(*a*) *Benzol and phenol derivatives*—Continued.					
		Per cent.			
Cresol calcium	P. hirsutus	Between 1 and 2.	Agar	8 days	Rumbold.
Do	Fomes annosus	Between 0.14 and 0.28.	do	4 to 7 weeks	Humphrey and Fleming.
Dinitro-p-cresol [$C_6H_2(NO_2)_2CH_3OH$].	Coniophora cerebella.	Under 0.1	do	8 to 10 days	Falck.
Sodium salt of—					
Dinitro-p-cresol, 21 per cent.[1]	do	Under 0.01	do	do	Do.
1:2 dinitro-o-cresol, 31 per cent.[1]	do	Under 0.003	do	do	Do.
4:6 dinitro-m-cresol, 59 per cent.[1]	do	Under 0.005	do	do	Do.
Gallic acid [$C_7H_6O_5+H_2O$]	do	Under 2	do	do	Do.
Gallotannic acid [$C_{14}H_{10}O_4$].	do	Under 1	do	do	Do.
Phenol [C_6H_5OH]	do	Under 0.1	do	do	Do.
Phenol, pure	Penicillium	0.15	do	4 weeks	J. M. Weiss.
O-nitrophenol [$C_6H_4NO_2OH$].	Coniophora cerebella.	Under 0.02	do	8 to 10 days	Falck.
P-nitrophenol	do	Under 0.01	do	do	Do.
Sodium salt of—					
O-nitrophenol, 63 per cent.[1]	do	Under 0.013	do	do	Do.
P-nitrophenol, 60 per cent.[1]	do	Under 0.025	do	do	Do.
2:4 dinitrophenol [$C_6H_3(NO_2)_2OH$].	do	Under 0.01	do	do	Do.
2:4 dinitrophenol, 33 per cent.[1]	do	Under 0.003	do	do	Do.
Salycilic acid [$C_6H_4OHCOOH$].	do	Under 0.1	do	do	Do.
Sodium picrate [$C_6H_2(NO_2)_3ONa$], 40 per cent.[1]	do	Under 0.04	do	do	Do.
Thymol [$C_6H_3CH_3C_3H_7OH$].	do	Under 0.01	do	do	Do.
(*b*) *Tars and creosotes.*					
Coal-tar creosote:					
Straight run, American (sp. gr. 1.049 at 15.5° C.).	Penicillium	0.15	Agar	4 weeks	J. M. Weiss.
5 per cent gum-arabic emulsion.	Polystictus versicolor.	0.25–0.40	do		Dean and Downs.
German (sp. gr. 1.09 at 15° C.).	Molds	0.2	do	4 weeks	J. M. Weiss.
Sp. gr. 1.048 at 60° C.	Fomes pinicola	0.225	do	4 to 6 weeks	Humphrey and Fleming.
5 per cent gum-arabic emulsion.	F. annosus	0.55	do	do	Do.
German (sp. gr. 1.062 at 38° C.).	Coniophora cerebella.	Under 0.125	do	8 days	Rumbold.
Do	Lenzites sepiaria	Under 0.125	do	do	Do.
Do	Polystictus versicolor.	Under 0.25	do	do	Do.
Do	P. hirsutus	Under 0.25	do	do	Do.
Carbolineum:					
Avenarius (sp. gr. 1.126 at 16.5° C.).	Fomes annosus	5.25	do	4 to 10 weeks	Humphrey and Fleming.
Do	F. pinicola	0.30	do	5 to 6 weeks	Do.
S. P. F. (sp. gr. 1.127 at 16° C.).	F. annosus	4.5	do	5 to 8 weeks	Do.
Coal-tar creosote:					
With bases, acids, and solid hydrocarbons removed.	Penicillium	0.85	do	4 weeks	J. M. Weiss.
With 20 per cent tar acids added.	do	0.15	do	do	Do.
With 20 per cent pure naphthalene added.	do	0.95	do	do	Do.
With 5 per cent filtered tar added.	do	0.45	do	do	Do.

[1] Toxic value based on 100 per cent pure salt.

TABLE IV.—*Toxicity of various preservatives to certain wood-destroying and other fungi*—Continued.

Toxic substance.	Organism.	Toxic point.	Culture medium.	Duration of test.	Investigator.
B.—ORGANIC COMPOUNDS—continued.					
(*b*) *Tars and creosotes*—Continued.					
Coal-tar creosote—Contd.		*Per cent.*			
With 10 per cent filtered tar added.	Penicillium	0.30	Agar	4 weeks	J. M. Weiss.
With 20 per cent filtered tar added.	do	0.60	do	do	Do.
Coal tar (undistilled, sp. gr. 1.194 at 15.5° C.).	Molds	Between 1.5 and 2.	do	do	Do.
Coal-tar creosote:					
Fraction I (sp. gr. 0.934 at 60° C., 78.3 per cent distills below 215° C.).	Fomes annosus	0.30	do	4 to 6 weeks.	Humphrey and Fleming.
Do	F. pinicola	0.225	do	do	Do.
Fraction II (sp. gr. 1.003 at 60° C., 9 per cent distills below 205° C., 95.9 per cent below 287° C.).	F. annosus	0.225	do	5 to 9 weeks.	Do.
Do	F. pinicola	0.15	do	4 to 5 weeks.	Do.
Fraction III (sp. gr. 1.045 at 60° C., 73.7 per cent distills below 295° C.).	F. annosus	0.325	do	4 to 8 weeks.	Do.
Do	F. pinicola	0.125	do	4 to 7 weeks.	Do.
Fraction IV (sp. gr. 1.088 at 60° C., 54.3 per cent distills between 285° and 320° C.).	F. annosus	3.3	do	4 to 6 weeks.	Do.
Do	F. pinicola	0.125	do	5 to 8 weeks.	Do.
Fraction V (10 per cent distills above 320° C., 36.6 per cent hard residue).	F. annosus	33	do	4 to 6 weeks.	Do.
Do	F. pinicola	7.8	do	5 to 8 weeks.	Do.
Fraction below 235° C.	Penicillium	0.35	do	4 weeks	J. M. Weiss.
Fraction between 235°–272° C.	do	0.25	do	do	Do.
Fraction above 272° C.	do	Between 4 and 4.5.	do	do	Do.
Anthracene, pure	do	Above 10	do	do	Do.
Naphthalene, pure	do	Between 9 and 10.	do	do	Do.
Quinolin, pure	do	0.10	do	do	Do.
Paraffin, pure	do	Above 10	do	do	Do.
Water-gas tar distillate:					
Fraction between 170°–340° C.	Polystictus versicolor	0.40	do		Dean and Downs.
Fraction between 170°–315° C. (sp. gr. 1.024 at 15.5° C.).	Penicillium	2	do	4 weeks	J. M. Weiss.
Fraction between 210°–315° C. (sp. gr. 1.053 at 15.5° C.).	do	Above 10	do	do	Do.
United Gas Improvement Co. oil (sp. gr. 1.058 at 60° C.).	Fomes annosus	Above 40	do	4 to 8 weeks.	Humphrey and Fleming.
Do	F. pinicola	Above 40	do	4 to 5 weeks.	Do.
Sp. gr. 0.995 at 60° C.	F. annosus	About 0.65	do	5 to 8 weeks.	Do.
Wood creosote (softwood, sp. gr. 1.052 at 60° C.—about 8 per cent H_2O).	do	0.65	do	4 to 7 weeks.	Do.
Do	F. pinicola	0.20	do	4 to 6 weeks.	Do.
Wood tar (hardwood, sp. gr. 1.195 at 60° C.).	F. annosus	1.25	do	do	Do.
Do	F. pinicola	0.75	do	4 to 5 weeks.	Do.
Semiasphaltic petroleum (sp. gr. 0.973 at 15.5° C.: distills above 315° C.).	Penicillium	Above 10	do	4 weeks	J. M. Weiss.

In conclusion, the writers wish to emphasize that any scale of toxicities derived from Petri-dish tests on the usual nutrient agar or

gelatin media, even when the tests are conducted under exactly similar conditions, do not necessarily represent the true relative toxic values of the different compounds, for the interaction between the toxic compounds, the nutrient substances contained in the media, and the plant protoplasm is variable and more or less specific for each combination.

Also, the reader should keep before him the fact that toxicity alone is not the sole criterion in judging the service value of a preservative, and a direct application of these data to that end would in many cases lead to very erroneous conclusions.

In many cases it is possible to overcome such unfavorable properties in a preservative as high solubility in water by placing the treated timber under conditions less exposed, and such timbers treated with soluble preservatives, such as sodium fluorid, zinc chlorid, and copper sulphate, should behave more or less according to the toxic ratios represented. The same should apply to oils of similar volatile and soluble properties placed under approximately similar service conditions.

Not all preservatives are adapted to the same uses, and in testing their service value these primary facts should be given full consideration. We have long been in the habit of taking as the standard test of the efficiency of a substance its ability to protect timber exposed to such extreme conditions as are railway ties, telephone poles, posts, exterior building timbers, etc. This standard is very often too severe, and for this reason preservatives should be grouped according to the conditions under which they are to be exposed.

SUMMARY.

A survey of the work of various investigators on the action of different toxic substances on the higher and lower forms of plant life discloses a marked difference in behavior. The action of toxic agents appears to be specific, being highly poisonous to certain organisms and only moderately so to others.

Very dilute concentrations ordinarily produce a stimulative effect.

Among the fungi, as a rule, the common molds are more resistant to poisons than the true wood-destroying fungi, and even among the latter group the different species show a great difference in susceptibility.

The chemical and physical composition of the media supporting the growth of the fungi determines, to a large extent, their development. The presence of certain kinds of insoluble matter or of such organic compounds as sugars and proteid materials, with which the toxic agents may possibly react, often introduces a considerable element of error when testing the toxic value of a substance by mixing it with nutrient agar or gelatin media.

Temperature is also an elemental factor in the growth of fungi, and there is an optimum for each organism, often lying within a very narrow range. The growth activities of fungi probably bear a close relation to the resistance offered toward toxic agents.

The toxic elements or radicals in a compound are often difficult to determine. In the case of heavy metallic salts, it is the metal ion; in the case of strong inorganic acids, the hydrogen ion is said to be the important element; in the fluorin compounds, fluorin is the determining agent; in the case of certain phenols, the introduction of halogen, alkyl, or nitro groups is said to increase toxicity.. Even in the case of isomeric compounds the grouping of the radicals plays an important part.

The Petri-dish method of determining the toxicity of a substance offers a ready procedure which gives indicatory results in a short time. On account of certain sources of error, some inaccuracies must be admitted, although the methods employed by the writers obviate many of these.

The results of tests on 18 wood preservatives at the Forest-Products Laboratory, against two wood-destroying fungi, *Fomes annosus* Fr. and *F. pinicola* (Sw.) Fr., are given. The preservatives act in a considerably different manner on these two organisms, the former being, as a rule, far more resistant.

The tests show that for these two organisms the following quantities of preservative per cubic foot of culture medium used are sufficient to inhibit all growth:

FOR FOMES ANNOSUS.

	Pounds.
Coal-tar creosote, Fraction II	0.14
Sodium fluorid	.16
Cresol calcium	0.09–.18
Coal-tar creosote:	
Fraction I	.19
Fraction III	.20
Zinc chlorid	.31
Coal-tar creosote, grade C	.34
Water-gas tar distillate (sp. gr. 0.995)	.41
Wood creosote	.41
Hardwood tar	.78
Coal-tar creosote, Fraction IV	2.06
S. P. F. carbolineum	2.8
Avenarius carbolineum	3.27
Coal-tar creosote, Fraction V	20.59
Copperized oil	25
United Gas Improvement Co., 1.07 oil	Over 25
None-Such Special	Over 25
Sapwood antiseptic	Over 25

FOR FOMES PINICOLA.

	Pounds.
Coal-tar creosote:	
Fraction III	0.08
Fraction IV	.08
Fraction II	.09
Sodium fluorid	.09
Wood creosote	.13
Coal-tar creosote:	
Grade C	.14
Fraction I	.14
Avenarius carbolineum	.19
Zinc chlorid	.47
Hardwood tar	.47
Coal-tar creosote, Fraction V	4.87
Copperized oil	Over 25
United Gas Improvement Co., 1.07 oil	Over 25
None-Such Special	Over 25

BIBLIOGRAPHY.

1. Bokorny, Thomas. Einwirkung von Metallsalzen auf Hefe und andere Pilze. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 35, No. 6/10, p. 118–197, 1912.
2. Brooks, Charles. Temperature and toxic action. *In* Bot. Gaz., v. 42, no. 5, p. 359–375, 1906.
3. Clark, J. F. On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. *In* Bot. Gaz., v. 28, 1899–1900: no. 5, p. 289–327; no. 6, p. 378–404.
4. Dean, A. L., and Downs, C. R. Antiseptic tests of wood-preserving oils. *In* Orig. Commun. 8th Internat. Cong. Appl. Chem., v. 13, Sect. 6a, p. 103–110 [1912].
5. Falck, Richard. Wachstumgesetze, Wachstumfaktoren und Temperaturwerte der holzzerstörenden Mycelien. *In* Möller, Alfred. Hausschwammforschungen. Heft 1, p. 53–154, 1907.
6. ——— Die Lenzitesfäule des Coniferenholzes, eine auf kultureller Grundlage bearbeitete Monographie der Coniferenholz bewohnenden Lenzites-Arten. *In* Möller, Alfred. Hausschwammforschungen. Heft 3, 234 pp., 24 fig., 7 pl., 1909.
7. ——— Die Merulius-fäule des Bauholzes. Neue Untersuchungen über Unterscheidung, Verbreitung, Entstehung und Bekämpfung des echten Hausschwammes. *In* Möller, Alfred. Hausschwammforschungen. Heft 6, 405 p., 73 fig., 17 pl., 1912.
8. Fitch, Ruby. The action of insoluble substances in modifying the effect of deleterious agents upon the fungi. *In* Ann. Mycol., v. 4, no. 4, p. 313–322, 1906.
9. Fred, E. B. Über die Beschleunigung der Lebenstätigkeit höherer und niederer Pflanzen durch kleine Giftmengen. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 31, No. 5/10, p. 185–245, 1 fig., 1911.
10. Guéguen, F. Action de divers antiseptiques sur le Penicillium glaucum. *In* Bul. Soc. Mycol. France, t. 15, fasc. 1, p. 15–23, 1899.
11. Harvey, H. W. The action of poisons upon Chlamydomonas and other vegetable cells. *In* Ann. Bot., v. 23, no. 90, p. 181–187, 2 fig., 1909.
12. Heald, F. D. On the toxic effect of dilute solutions of acids and salts upon plants. *In* Bot. Gaz., v. 22, no. 2, p. 125–153, pl. 7, 1896.
13. Hoffmann, Karl. Wachstumverhältnisse einiger holzzerstörender Pilze. *In* Ztschr. Naturw., Bd. 82, p. 35–128, 1910. *Also reprinted* as Inaugural Dissertation, Konigsberg in Pr.
14. Kahlenberg, Louis, and True, R. H. On the toxic action of dissolved salts and their electrolytic dissociation. *In* Bot. Gaz., v. 22, no. 2, p. 81–124, 1896.
15. Kellerman, K. F. The excretion of cytase by Penicillium pinophilum. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 118, p. 29–31, 2 fig., 1913.
16. Le Renard, Alfred. Influence du milieu sur la résistance du Pénicille crustacé aux substances toxiques. *In* Ann. Sci. Nat., Bot., s. 9, t. 16, no. 4/6, p. 277–336, 1912.
17. McClintic, T. B. Chloride of zinc as a deodorant, antiseptic, and germicide. U. S. Public Health and Marine Hosp. Serv. Hyg. Lab. Bul. 22, 24 p., 1905.

18. Malenković, Basilius. Zur Lehre und Anwendung der Holzkonservierung in Hochbaue. Mitt. Gegenst. Artil. u. Geniew., [Vienna], Jahrg. 35, 1904, p. 311–333.

19. ——— Die Holzkonservierung im Hochbaue mit besonderer Rücksichtnahme auf die Bekämpfung des Hausschwammes, 301 p. Vienna, 1907. For French translation of first part *see* Ann. Agron. s. 3, ann. 6, sem. 1, No. 3, p. 161–211, 1911.

20. ——— Über den Zusammenhang zwischen der Zufuhr von Antiseptikum und der Lebensdauer bei imprägnierten Holzmasten. *In* Elektrotek. Ztschr., Jahrg. 34, 1913, Heft 16, p. 436–439.

21. Netzsch, J. Die Bedeutung der Fluorverbindungen für die Holzkonservierung. *In* Naturw. Ztschr. Forst u. Landw., Jahrg. 8, 1910, Heft 8, p. 377–389.

22. Ono, N. Zur Frage der chemischen Reizmittel. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 9, No. 5/10, p. 154–160, 1902.

23. Pulst, Carl. Die Widerstandfähigkeit einiger Schimmelpilze gegen Metallgifte. *In* Jahrb. Wiss. Bot. [Pringsheim], 1902, Bd. 37, Heft 2, p. 205–263.

24. Raulin, Jules. Études chimiques sur la végétation. *In* Ann. Sci. Nat. Bot., s. 5, t. 11, p. 93–299. Also reprinted, Paris, 1870.

25. Rumbold, Caroline. Über die Einwirkung des Säure- und Alkaligehaltes des Nährbodens auf das Wachstum der holzzersetzenden und holzverfärbenden Pilze; mit einer Erörterung über die systematischen Beziehungen zwischen Ceratostomella und Graphium. *In* Naturw. Ztschr. Forst u. Landw., Bd. 9, Heft 10, p. 429–465, 22 fig., 1911.

26. Seidenschnur, F. Wood preservation, 1911. Advertising pamphlet published by Hülsberg & Co.

27. Thiele, R. Die Temperaturgrenzen der Schimmelpilze in Verschiedenen Nährlösungen, 37 p., 6 pl. Leipzig, 1896.

28. True, R. H. The toxic action of a series of acids and of their sodium salts on Lupinus albus. *In* Amer. Jour. Sci., s. 4, v. 9, no. 51, p. 183–192, 1900.

29. ——— and Gies, W. J. On the physiological action of some of the heavy metals in mixed solutions. *In* Bul. Torrey Bot. Club, v. 30, no. 7, p. 390–402, 1903.

30. ——— and Hunkel, C. G. The poisonous effects exerted on living plants by phenols. *In* Bot. Centbl., Bd. 76, 1898: No. 9, p. 289–295; No. 10, p. 321–327; No. 11, p. 361–368; No. 12, p. 391–398.

31. ——— and Oglevee, C. S. The effect of the presence of insoluble substances on the toxic action of poisons. *In* Bot. Gaz., v. 39, no. 1, p. 1–21, 2 fig., 1905.

32. Weiss, H. F. Tests to determine the commercial value of wood preservatives. A progress report. *In* Orig. Commun. 8th Internat. Cong. Appl. Chem., v. 13, Sect. 6a, p. 279–300, 5 fig. [1912].

33. Weiss, J. M. The action of oils and tars in preventing mould growth. *In* Jour. Soc. Chem. Indus., v. 30, no. 4, p. 190–191, 1911.

34. ——— The antiseptic effect of creosote oil and other oils used for preserving timber. *In* Jour. Soc. Chem. Indus., v. 30, no. 23, p. 1348–1353, 1911.

○